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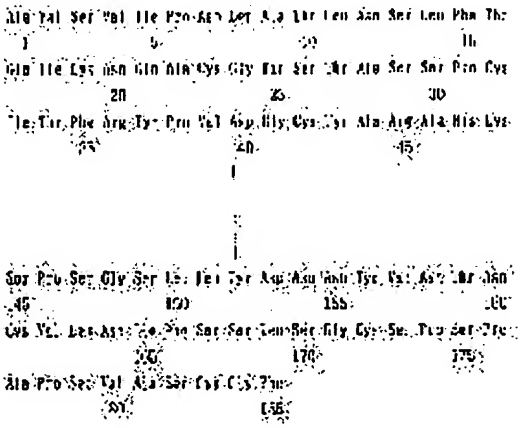
(54) NEW PROTEIN DEAMIDASE, GENE ENCODING THE SAME, ITS PRODUCTION AND USE

(57)Abstract:

PROBLEM TO BE SOLVED: To provide a new protein deamidase that act on glutamine in the protein to catalyze deamidation reaction and is useful for modification of proteins, functional modification of proteins, or functional modification of foods.

SOLUTION: This is a new polypeptide that has the amino acid sequence described in the formula or in which one or a plurality of amino acid residues are deleted from and/or added to and/or inserted into the polypeptide. The polypeptide is a new enzyme that directly acts on the amide groups in a protein to effect deamidation without cleavage of the peptide linkage and crosslinking of protein and is useful for modification of protein, functional modification of protein and food products. This enzyme is obtained by culturing a bacterium in Cytophagales or Actinomycetes in a nutrient medium to allow the



microorganism to produce the objective deamidase and collecting the enzyme from the culture mixture.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention acts on the side-chain amide group in a new enzyme, i.e., protein, and relates to the new enzyme which has the operation which separates a side-chain carboxyl group and ammonia, and its manufacturing method. Furthermore, the bacteria classified into a detail at SHITOFAGARESU (Cytophagales) or bitter taste chino my SETESU (Actinomycetes), In a detail, more A KURISEO bacterium (Chryseobacterium) group, The Flavobacterium (Flavobacteium) group, an en PEDOBAKUTA (Empedobacter) group, The Sphingobacterium (Sphingobacterium) group, It belongs to an AUREO bacterium (Aureobacterium) group and a MIROIDESU (Myroides) group. Cultivate the strain which has the enzyme productivity which has the property which carries out deamidation of the amide group in protein to a culture medium, this enzyme is made to produce, and it is related with the manufacturing method of the enzyme which has the property which carries out deamidation of the amide group in the protein characterized by extracting this enzyme from a culture. Furthermore, this invention relates to the qualification approach of the protein using the new enzyme which carries out a direct action to the amide group in protein. Furthermore, this invention cultivates the transformant which introduced the vector containing the gene which carries out the code of the enzyme which has the property which carries out deamidation of the amide group in protein, and this enzyme, and this gene, and this vector, and a transformant to a culture medium, makes this enzyme produce, and relates to the manufacturing method of the enzyme which has the property which carries out deamidation of the amide group of the protein characterized by extracting this enzyme from a culture.
[0002]
[Description of the Prior Art] Glutaminase/asparaginase is enzymes which carry out hydration of a glutamine/the asparagine, and are used as glutamic acid/aspartic acid, and ammonia, and it is known well that this will be obtained from animals and plants and a microorganism. However, this enzyme is an enzyme which acts on the glutamine/asparagine of isolation specifically, and cannot carry out deamidation of the glutamine/the asparagine in a peptide. Furthermore, deamidation of the gamma/beta-amide group of the glutamine/asparagine in protein with bigger molecular weight than a peptide cannot be carried out.
[0003] Moreover, there is transglutaminase as an enzyme which acts on the amide group which exists in a peptide condition. This enzyme makes an acyl acceptor the amino group of an acyl donator and primary amine for the amide group of a peptide linkage glutamine, and carries out the catalyst of the arch forming by covalent-bond-installation of an amine compound, and the epsilon-(gamma-glutamyl) lysine-peptide linkage between the glutamine in protein, and lysine both residue to protein. The reaction which crosslinking reaction will occur if it is made to act on the usual protein since this enzyme is originally acyl transferring enzyme as mentioned above, although water becoming an acyl acceptor when it is blocked, or neither an amine nor a lysine exists in the system of reaction, and deamidation of the glutamine residue in a peptide being carried out, and becoming glutamic acid is known, and deamidates protein is not produced, but it differs from the enzyme of this invention.
[0004] Moreover, about the enzyme which acts and carries out deamidation to the glutamine combined in a peptide, it is a bacillus. The enzyme of the SAKYU lance (Bacillus circulans) origin, Peptideglutaminase I and Peptideglutaminase II are known. it is the enzyme which . by which it is known that the former will act on the glutamine residue located in the C terminal of a peptide, and the latter will act on the glutamine residue in a peptide, however these enzymes do not act on giant-molecule protein, but acts only on a low-molecular peptide [M.Kikuchi, H.Hayashida, E.Nakano, and K.Sakaguchi, Biochemistry, ten volumes, and 1222-1229 (1971)].
[0005] Although the attempt which makes these enzymes (PeptideglutaminaseI and II) act on the protein of the macromolecule instead of a low-molecular peptide has been made by two or more researches On . concrete target with which it is shown clearly that those enzymes do not act on giant-molecule protein substantially, but act only on a proteolysis peptide Gill and others -- milk casein and HOEI protein -- receiving -- not only a native condition but after denaturation -- also setting -- PeptideglutaminaseI and II -- it has reported that neither acts. them -- moreover, although

only Peptideglutaminase II acted as a result of examining the operation nature to the hydrolyzate of those protein, it has reported acting only on a with a molecular weight of 5000 or less peptide (B.P.Gill, A.J.O'Shaughnessey, P.Henderson and D.R.Headon, Ir.J.Food Sci.Technol., nine volumes, and 33-41 (1985)). Although same examination was performed by Hamada and others using soybean protein, coincidence with Gill's and others result is obtained. That is, it is reported that the rate of deamidation to an soybean peptide (Peptone) does not act substantially to soybean protein to 24.4-47.7% (0.4-0.8%) (J.S.Hamada, F.F.Shih, A.W.Frank and W.E.marshall, J.Food Science, 53 volumes, No. 2, and 671-672 (1988)).

[0006] The possibility of the existence of the enzyme which carries out deamidation of the protein into a vegetable seed is reported (I. A.Vaintraub, l.V.Kotova, R.Shara, FEBS Letter, 302 volumes, and 169-171 (1992)). However, in this report, although isolation of ammonia is observed from protein using a partial refined material, it is clear that it is not what proves existence of the enzyme indicated by this invention for the reason explained below. With namely, the point of using the partial refined material, the point that it is not checked that protease activity does not exist, and the point that it is not checked that molecular weight change of the protein matrix after a reaction has not arisen Not an operation of one enzyme but two or more enzymes, for example, a protease, the amino acid isolated from protein by the peptidase, Possibility that glutaminase/asparaginase deamidates a glutamine/asparagine, and ammonia has separated, or possibility that deamidation of the glutamine content low-molecular peptide produced similarly is carried out with the peptide glutaminase Mr. enzyme is left behind. Or possibility that deamidation is carried out by the double reaction of a protease again cannot be denied, either. It should mention specially that it is specified that the glutaminase activity which especially acts on the glutamine of isolation in the product made from a part used during the above-mentioned report, and separates ammonia exists. Thus, the report proving the existence was unprecedented by refining even single protein, carrying out simple interest of the gene further, and making it discovered about the enzyme which carries out a macromolecule protein operation and carries out the catalyst of the deamidation reaction.

[0007] If the glutamine and asparagine residue in protein are generally deamidated and a carboxyl group is produced, the negative charge of the protein will increase and, as a result, the fall of the isoelectric point and the hydration force will increase. Furthermore, the fall of the interaction between the protein by the rise of the electrostatic rebounding force, i.e., the fall of meeting nature, is brought about. Proteinic fusibility and water-dispersion increase greatly by these change. Moreover, the increment in a proteinic negative charge unfolds folding of the protein, higher order structure is changed, and the hydrophobic field buried in the interior of a molecule is exposed on a molecule front face. Therefore, deamidation protein has amphiphilic, and serves as an ideal surfactant, and the proteinic emulsification force, emulsion stability, foamability, and its foam stability improve greatly.

[0008] Thus, proteinic deamidation brings about improvement in proteinic various functional characteristics, and the application of the protein is increased by leaps and bounds. (for example, Molecular Approaches to Improving Food Quality and Safety, D.Chatnagar and T.E.Cleveland, eds., Van Nostrand Reinhold, New York, 1992, p.37) .

[0009] For this reason, the method of deamidating protein was studied old more briskly, and has considered many approaches. As an approach of making it deamidate protein chemically, there was a mild acid or a mild alkali treatment method under a high temperature service etc. Generally the glutamine in protein and the amide group of asparagine residue are hydrolyzed by an acid or the base. However, this reaction is nonspecific and is accompanied also by cutting of peptide linkage under strong acid and strong-base conditions. Moreover, it is accompanied also by proteinic denaturation and results in spoiling the functionality of the protein.

[0010] then, many things are devised in order to restrict these reactions that are not desirable -- having -- mild acid treatment (For example) J. W.Finley and J.Food Sci.40, 1283, 1975 (for example); C.W.Wu, S.Nakai, and W.D.Powie, J.Agric.Food Chem., 24, 504, 1976, etc. and mild alkali treatment A.Dilollo, I.Alli, C.Biloarders, N.Barthakur, J.Agric.Food Chem., 41.24, 1993, etc. were devised. moreover As an acid Sodium dodecyl sulfate (F.) [F.Shih] Use and A.Kalmar, J.Agric.Food Chem., 35, 672, 1987, cation exchange resin (F.F.Shih, J.food Sci., 52, 1529, 1987), etc. as a catalyst, or Or the high-temperature-processing method (J.Zhang, T.C.Lee, andC.-T.Ho, J.Agric.Food Chem., 41, 1840, 1993) under a low-water-flow part etc. has been tried again.

[0011] However, it was difficult to restrict cutting of peptide linkage completely by any approach. Cutting of peptide linkage brings [it not only checks improvement in the functionality of the protein expected by deamidation, but] about generation of bitterness and is not desirable. Moreover, compared with an acid-treatment method, there was also a fault which lysinoalanine with racemization of amino acid or toxic misgiving produces by the efficient alkali treatment method.

[0012] On the other hand, in order to conquer the trouble of an above-mentioned chemistry method, some proteinic enzyme-deamidating methods have also been tried. The protease approach under high pH (pH10) conditions (A.Kato, A.Tanaka, N.Matsudomi, and K.Kobayashi, J.Agric.Food Chem., 35, 224, 1987), The transglutaminase method (M.Motoki, K.Seguro, A.Nio, and K.Takinami, Agric.Biol.Chem., 50, 3025, 1986), Although the three approaches of the peptide glutaminase method (J. S.Hamada, and W.E.Marshall, J.Food Sci., 54, 598, 1989) had been considered, all had a fault.

[0013] By the protease method, cutting of the peptide linkage which is the original reaction was not avoided probably. It is as above-mentioned that cutting of peptide linkage is not desirable.
[0014] Moreover, by the transglutaminase method, in order to press down the crosslinking reaction by formation of the isopeptide bond between the glutamine which is the original reaction, and a lysine, epsilon-amino group of lysine residue needed to be protected chemically beforehand. When a food grade etc. is presented with deamidation protein, after protecting by the SHITORAKONIRU radical which is a reversible protective group, deamidation of the glutamine had to be carried out, the aftercare radical had to be removed, and the SHITORAKONIRU acid and deamidation protein which separated further had to be separated. These processes increase a manufacturing cost greatly and it is clear that its it is far from utilization.
[0015] On the other hand, by the peptide glutaminase method, since it was the enzyme with which this enzyme hardly acts on protein, but acts only on a low-molecular peptide, it could not be made to act on raw protein, but protein hydrolysate needed to be used.
[0016] Thus, in the purpose which deamidates protein, although it is one of the greatest advantages which endures a physical method, since the suitable enzyme for side reaction not following, and acting on macromolecule protein, and deamidating did not exist, the present condition is the reaction selectivity which originally originates in the high substrate specificity which an enzyme's has in enzymatic process being chemical, and not put in practical use.
[0017] thus -- although proteinic deamidation is the outstanding embellishing method which brings about functional big improvement -- the conventional chemistry method and enzymatic process -- there is a fault by any approach and utilization was not progressing.
[0018]
[Means for Solving the Problem] Therefore, this invention persons ask a cheap microorganism for the source of supply of the enzyme which carries out a direct action to the amide group in the condition of having combined with protein and which carries out deamidation to it. The new strain which belongs to the KURISEO bacterium (Chryseobacterium) group which this invention persons more newly than the inside of soil separated as a result of repeating screening wholeheartedly It found out producing the enzyme which has the operation which carries out a direct action to the amide group combined in protein, and accompanies by them and carries out deamidation of association of a peptide, and the proteinic bridge formation, and this invention was completed. The enzyme which has an above-mentioned operation in this specification is called the protein deamidase.
[0019] Furthermore, this invention persons chose the strain belonging to the KURISEO Bacterium from the type culture at random, and considered production of the protein deamidase by the strain. Consequently, the productivity was checked by all strain and the production was checked by other strain, for example, Flavobacterium (Flavobacteium) group, en PEDOBAKUTA (Empedobacter) groups and Sphingobacterium (Sphingobacterium) groups, AUREO bacterium (Aureobacterium) groups, and MIROIDESU (Myroides) groups. Each of such strain is bacteria classified into SHITOFAGARESU (Cytophagales) or bitter taste chino my SETESU (Actinomycetes), and each of a KURISEO bacterium (Chryseobacterium) group, en PEDOBAKUTA (Empedobacter) groups, Flavobacterium (Flavobacteium) groups, and MIROIDESU (Myroides) groups is bacteria classified into FURABOBAKUTERIACHIE (Flavobacteriaceae).
[0020] Furthermore, this invention persons isolated and refined the protein deamidase, determined the base sequence of the gene which carries out the code of this protein deamidase, and checked that it was possible to manufacture the protein deamidase using the transformant which introduced the vector containing this gene further.
[0021] Namely, the method of producing the protein deamidase using the microorganism to which this invention produces the protein deamidase, The qualification approach of the protein using the protein deamidase, the constituent for qualification of the protein which contains the protein deamidase as an active principle, The approach of improving the functionality of the protein using the protein deamidase, the method of improving the functionality of the food using the protein deamidase, It is related with the method of improving the extraction efficiency of the protein using the protein deamidase, and/or a peptide, and the approach of controlling the reaction of the transglutaminase using the protein deamidase.
[0022] Furthermore, this invention cultivates the transformant which introduced the recombination vector containing the gene which carries out the code of the protein deamidase and this enzyme, and this gene, and this vector, and this transformant to a culture medium, makes the protein deamidase produce, and relates to the manufacturing method of the protein deamidase characterized by extracting the protein deamidase from a culture.
[0023] The protein deamidase of this invention may be effective also to the amide group which especially the site of action is not limited to them, and was combined with other amino acid residue in protein although it was effective in the amide group of asparagine residue and glutamine residue at least. In addition, in this application specification, protein may not be limited to a protein simple substance and may be conjugated protein with sugar, a lipid, etc. And although especially the molecular weight of the protein is not limited, the range of it is usually 10,000-2,000,000 preferably more than 5000 (50 residue).

[0024] Moreover, the protein deamidase of this invention can be used for deamidation also to the peptides which have an amide group besides protein, those derivatives, etc. As a peptide, the thing of 2-50 is mentioned and the number of amino acid residue is usually suitable for what is used for the nutrition improvement agent etc. as an application. That is, the protein deamidase of this invention can make even protein of a macromolecule a substrate from more than the dipeptide containing a polypeptide. In addition, the vocabulary the "polypeptide" of this application specification contains protein.
[0025]
[Embodiment of the Invention] The microorganism which produces the protein deamidase of this invention is the following, and can be made and screened. That is, by inoculating the suspension of soil into the liquid medium for separation which contains Z-Gln-Gly as an only nitrogen source, enrichment culture is performed, the culture medium is applied to the same monotonous agar medium for separation, and the grown colony is chosen and gathered. Such strain can be cultivated by the suitable liquid medium, and the strain which has ammonia isolation activity can be chosen from Z-Gln-Gly.
[0026] Thus, about the selected strain, a protein deamidase production microorganism can be screened by making ammonia isolation activity into an index, being able to use casein as a substrate further.
[0027] Thus, the screened strain was identified the KURISEO Bacterium according to the manual OBU data MINETEIBU bacteriology of bar G work. Furthermore, it checked that the protein deamidase was produced by the assay same also about the strain chosen from the type culture of the KURISEO Bacterium as random as ****. As a more concrete strain, for example KURISEO bacterium GUREUMU () [Chryseobacterium] gleumJCM2410, KURISEO bacterium indolo GENESU (Chryseobacterium indologenes) IFO14944, a KURISEO bacterium MENINGOSE petit cam () [Chryseobacterium] meningosepticumIFO12535, KURISEO bacterium ballast CHINAMU (Chryseobacterium balustinum) IFO15053, a KURISEO bacterium in dollar ceti cam () [Chryseobacterium] indolthticum ATCC27950, KURISEO bacterium SUKOFUSARUNAMU (Chryseobacterium scophthalnum CCM4109), etc. are mentioned.
[0028] Moreover, the place which performed screening with the same said of other microorganisms, As Flavobacterium and a more concrete strain, for example, Flavobacterium AKUATIRE (Flavobacterium aquatile) IFO15052, an en PEDOBAKUTA (Empedobacter) group, As a more concrete strain, for example, the en PEDOBAKUTA brevis (Empedobacter brevis) IFO14943, the Sphingobacterium (Sphingobacterium) group, As a more concrete strain **, for example, Sphingobacterium SUPIRICHIBORAMU () [Sphingobacterium] spiritivorumIFO14948, Sphingobacterium HEPARINAMU (Sphingobacterium heparinum) IFO12017, an AUREO bacterium (Aureobacterium) group, As a more concrete strain, for example, the AUREO bacterium ESUTERO aroma tee cam (Aureobacterium esteraromatidum) IFO3751 and a MIROIDESU (Myroides) group, Production of the protein deamidase was checked by the strain of MIROIDESU ODORATASU (Myroidesodoratus) IFO14945 as a more concrete strain.
[0029] In addition, this enzyme does not have, the activity, i.e., the transglutaminase activity, which carries out the catalyst of the iso peptide formation between proteinic glutamine residue and lysine residue, but known transglutaminase is distinguished. Moreover, it does not have, the activity, i.e., the protease activity, which hydrolyzes proteinic peptide linkage, but is distinguished also from a known protease.
[0030] Although any of liquid culture and solid culture are sufficient as cultivation of the strain for manufacturing the protein deamidase using each strain mentioned above, liquid culture is used preferably. It can carry out by [as being the following as liquid culture].
[0031] As long as it is the culture medium which can grow the microorganism which produces the protein deamidase as a culture medium which can be used, what kind of thing may be used. For example, nitrogen sources, such as carbon sources, such as a glucose, sucrose, a glycerol, a dextrin, molasses, and an organic acid, and also an ammonium sulfate, an ammonium carbonate, ammonium phosphate, ammonium acetate or a peptone, a yeast extract, corn steep liquor, casein hydrolysate, and a meat extract, and the thing which added the mineral salt of potassium salt, magnesium salt, sodium salt, phosphate, manganese salt, iron salt, zinc salt, etc. further can be used.
[0032] pH of a culture medium -- about 3-10 [for example,] -- desirable -- about seven to about eight -- preparing -- culture temperature -- usually -- about 10-50 degrees C -- desirable -- about about 20-37 degrees C -- one - 20 days -- desirable -- about a 3-12 day room -- it cultivates under aerobic conditions. As cultivation, shaking culture and the aerobic submerged culture method by the jar fermenter can be used.
[0033] The protein deamidase is isolated from the obtained culture medium with the usual means, the protein deamidase of this invention is obtained, and things are made. For example, various chromatographies, such as centrifugal separation, UF concentration, a salting-out, and ion exchange resin, are combined, in order to carry out isolation purification of the protein deamidase, it can process with a conventional method and the refined protein deamidase can be obtained from culture medium.
[0034] Furthermore, this invention is explained in full detail more concretely. That is, KURISEO bacterium

GUREUMU (Chryseobacterium gleum) JCM2410 which produces the protein deamidase and which was mentioned above as strain was used, it cultivated by the liquid medium, and production of the enzyme concerned, purification of this enzyme, and many properties of an enzyme were examined.

[0035] The bacillus of one platinum loop is taken from a fresh slant, by the following lactose culture medium, shaking culture is carried out for two - seven days, and 30 degrees C of centrifugal supernatant liquid are obtained after that.

Lactose medium composition (pH7.2)

Lactose (product made from the Wako Pure Chem industry) 0.5% poly peptone (product made from the Wako Pure Chem industry) 1.0%Na2HPO4 and H2O 0.17%KH2PO4 0.025%MgSO4 and 7H2O 0.025 %FeSO4 and 7H2O 0.005 % culture progress was shown in drawing 1 .

[0036] The purification approach of an enzyme carried out centrifugal separation (for 12000rpm, 4 degrees C, and 20 minutes) of the culture medium after culture termination, obtained supernatant liquid as crude enzyme liquid, processed it with UF concentration (SEP-0013), a salting-out, FENIRU sepharose, and the SEFA seal S-100, and refined the enzyme. The process of purification is shown in Table 1.

[0037]

[Table 1]

	総蛋白量 mg	総活性 U	比活性 U/mg	回収率 %
培養液	83.50	16.53	0.198	100
ＵＦ濃縮	11.78	13.17	1.12	79.7
塩析	4.10	10.03	4.09	60.7
ﾌｪﾆｰﾙｾﾌｧﾛｰｽ	0.187	3.10	16.6	18.7
ｾﾌｧｼｰﾙS-100	0.073	2.26	31.1	13.7

[0038] In addition, measurement of enzyme activity followed as follows and used Z-Gln-Gly and casein as a substrate.

[0039] The activity-measurement approach: Add 10micro of enzyme solutions l to 100micro [of 176mM phosphate buffer solutions] (pH6.5) l containing 10 mM Z-Gln-Gly, add 100micro of trichloroacetic-acid solutions l 12%, and suspend 37 degrees C of reactions, after incubating for 60 minutes. After carrying out centrifugal separation (for 15000rpm, 4 degrees C, and 5 minutes), it measures using F-kit ammonia (Boehringer Mannheim make) as follows about supernatant liquid (A1). Water is independently used instead of an enzyme solution, and it measures similarly (A2).

[0040] F-kit ammonia 100microl 10micro of supernatant liquid l and 190micro of water l are added to a reagent 2, and the absorbance of 340nm using 100microl after neglect (E1) is measured for 5 minutes at a room temperature. After adding the reagent 3 (glutamate dehydrogenase) of 1.0microl to the remaining 200microI, and leaving it in a room temperature for 20 more minutes, the absorbance of 340nm of the remaining 200microl (E2) is measured. The amount of enzymes which separates the ammonia of 1micromol per for 1 minute under the above-mentioned conditions is made into one unit, and it asks according to the following formulas.

u/ml=1.76x [A1(E1-E2)-A2 (E1-E2)]

It replaces with 10 mM Z-Gln-Gly as a substrate, activity is similarly searched for using casein (a HAMA stain, Merck Co. make) 1%, and it checks acting on the amide group combined with protein. Protease activity was measured by measuring the absorbance of 280 nm about the centrifugal supernatant liquid after a reaction halt to coincidence at this time. Protease activity made one unit the amount of enzymes which carries out 1OD unit rise under these conditions. It replaced with 10 mM Z-Gln-Gly as a substrate, and glutaminase activity was similarly measured using 10mM glutamine.

[0041] Moreover, transglutaminase activity was measured by the hydroxy sum acid process shown in the following which used Z-Gln-Gly as a substrate.

Reagent A 0.2M tris hydrochloric-acid buffer solution (pH6.0) 0.1M hydroxylamine 0.01M reduced glutathione 0.03M benzyloxycarbonyl - L-glutaminyl glycine Reagent B 3-N hydrochloric acid 12% trichloroacetic acid 5%FeCl3 and 6H2O (it dissolves in 0.1N HCl)

Let the mixed liquor of 1:1:1 of the above-mentioned solution be Reagent B. Reagent A 0.5ml is added to 0.05ml of enzyme liquid, and it mixes, and after adding reagent B0.5ml after a reaction for 10 minutes at 37 degrees C and performing formation of a reaction halt and Fe complex, the absorbance of 525nm is measured. Although it was made to react similarly using the enzyme liquid which carried out thermal inactivation beforehand as contrast, an absorbance is measured, and an absorbance difference with enzyme liquid is searched for. L-glutamic acid gamma-mono-hydroxamic acid was independently used instead of enzyme liquid, the calibration curve was created, the amount of the hydroxamic acid generated by said absorbance difference was calculated, and enzyme activity which generates the hydroxamic acid of 1micro mol in 1 minute was made into one unit.

[0042] In addition, the quantum of the proteinic quantum was carried out with the BCA protein assay kit (made in Pierce), using cow serum albumin as standard protein.
[0043] ** Measurement of molecular weight : they were 20kDa(s) in SDS-polyacrylamide gel electrophoresis.
[0044] ** Measurement of optimal pH : 10micro of enzyme liquid l which contains the protein deamidase of 0.32microg for 100(Z-Gln-Gly of 10mM is included) microl [40mM Britton-Robinson buffer solution (pH 3-12)] of each pH after a preheating for 5 minutes at 37 degrees C was added, it reacted for 60 minutes at 37 degrees C, and enzyme activity was measured. The result is shown in drawing 2 .
[0045] ** Measurement of optimum temperature : substrate solution (Z-Gln-Gly of 10mM is included) 100microl 10micro of enzyme solutions l which contain the protein deamidase of 1.21microg in [a 176mM phosphate buffer solution (pH6.5)] was added, it reacted for 60 minutes at each temperature, and enzyme activity was measured. The result is shown in drawing 3 .
[0046] ** Measurement of pH stability : process enzyme solution 22microl [40mM Britton-Robinson buffer solution (pH 3-12)] containing the protein deamidase of 0.75microg at 30 degrees C for 18 hours. The enzyme activity which remains after that was measured. The result is shown in drawing 4 .
[0047] ** Measurement of temperature stability : after leaving enzyme solution 43microl [50mM phosphate buffer solution (pH7.0)] containing the protein deamidase of 1.76microg at each temperature for 10 minutes, the enzyme activity which remains was measured. The result is shown in drawing 5 .
[0048] ** Substrate specificity : 10micro (10mU) of protein deamidase solutions l was added to 100micro of those solutions l, and it was made to react to them at 37 degrees C after mixing for 18 hours, using various protein (1%) solutions as a substrate. As contrast, water was added instead of enzyme liquid, it processed similarly, and the amount of the ammonia which separated was measured. The result of having deducted the amount of isolation ammonia in water addition from the amount of isolation ammonia in enzyme addition is shown in Table 2. Moreover, when SDS-PAGE was presented with a part of mixed liquor after reaction termination and having been compared with contrast, proteinic macromolecule-izing and proteinic depolymerize were not observed. This means that the transglutaminase of known [enzyme / this] and a prosthetic device are new enzymes distinguished.
[0049]
[Table 2]

蛋白質	遊離アンモニア (mM)
カゼイン	2.45
グルテン	1.85
大豆粉	0.10
コーンミール	0.12
β-ラクトグロブリン	0.65
オボアルブミン	0.24

[0050] ** Measurement of the isoelectric point : when measured by isoelectric point accumulation (600V, 4 degrees C, 48-hour energization) using ampholine, the isoelectric point of this enzyme was 10.2.
[0051] Subsequently, the qualification approach of the protein using the above-mentioned protein deamidase which is this invention is explained in full detail.
[0052] The protein deamidase of this invention is made to act on various protein. if you may be what kind of thing as long as it receives an operation of the above-mentioned enzyme as protein, for example, it is vegetable albumen and it is legumes, the protein of the cereals origin, and an animal protein -- tendons, such as blood protein, such as meat proteins, such as egg proteins, such as lactalbumins, such as casein and a beta lactoglobulin, and an ovalbumin, a myosin, and an actin, and serum albumin, gelatin, and a collagen, -- protein is raised. Moreover, you may be chemical or the enzyme-partial decomposition protein by a protease etc. and the chemical modification protein with various reagents by the acid, alkali, etc., and a synthetic peptide.
[0053] Although a reaction is presented with these protein matrices in the state of a solution, a slurry, or a paste, especially the concentration is not limited and is suitably chosen by the description and the condition that the target deamidation protein is desired. Moreover, the solution, slurry, or paste of this protein matrix may be an emulsion not only with a water solution but fats and oils, and salts, a saccharide, protein, perfume, a moisturizer, a coloring agent, etc. may be added further if needed.
[0054] although it is not that to which especially pH of the amount of enzymes, the time amount of a reaction, temperature, and a reaction solution etc. is limited as a reaction condition -- usually -- 1g of protein -- receiving -- 0.1 to 100 unit -- desirable -- one to 10 unit, and reaction temperature -- usually -- 5-80 degrees C -- desirable -- pH of 20-60 degrees C and a reaction solution -- usually -- 2-10 -- it is made to react preferably by 4-8 for 10 minutes to 24 hours

for 10 seconds to 48 hours Moreover, these conditions can be suitably changed according to the purity of the enzyme to be used, the class of protein matrix, purity, etc., and can be performed. Moreover, it also sets for the application of various this invention enzymes indicated below, and these reaction conditions are [0055]. [same] Thus, by making the protein deamidase of this invention act on various protein, direct deamidation of the amide group in protein can be carried out. Consequently, as for the produced deamidation protein, the fall of pI, the rise of the hydration force, and the rise of electrostatic repulsion are brought about with the increment in a negative charge. Furthermore, the rise of surface hydrophobicity is brought about by change of proteinic higher order structure. According to such effectiveness, an improvement of the functionality of protein, such as improvement in fusibility and dispersibility, improvement in foamability and foam stability, and improvement in emulsifiability and emulsion stability, is brought about.

[0056] Thus, the application in the food field mainly expands greatly the protein with which functionality has been improved. In the acescence which is the pH range of food especially usual in much vegetable albumen, since functionality, such as fusibility, dispersibility, and emulsifiability, was scarce, the use to acid drinks, such as much food, for example, a coffee whitener, and juice, a dressing, mayonnaise, a cream, etc. was restricted. However, by deamidating vegetable difficulty solubility protein, such as wheat gluten, by this invention, for example, fusibility and dispersibility increase, and it becomes usable to such food which did not fit use until now, and can be used also as tempura powder with high dispersibility.

[0057] Moreover, this enzyme can be used also for reforming of the dough in bread-making and confectionery. For example, the dough with a high gluten content had the low extensibility, and there was a problem also in the volume and quality of a pan which there is a problem and were done in the handling nature and the mechanical characteristic of dough. By deamidating gluten with this enzyme, an extensibility can improve and these problems can be solved. Moreover, deamidation gluten also shows the effectiveness as an emulsifier and bread-making properties' [, such as keeping nature and SOFUTONESU,] improves. since the dough which furthermore contains deamidation gluten excels [plasticity] in the extensibility low -- a cracker, a pith blanket, Cookie, and pizza -- or it is suitable to manufacture of crust of a pie, and this enzyme can be used also for these manufactures. For this application, the enzyme of this invention is usually preferably mixed by 0.1 to 150 unit, and the usual approach 0.01 to 10000 unit to the whole quantity of the dough which consists of wheat flour, water, etc.

[0058] Furthermore, by processing according the protein used as causes, such as allergy resulting from the protein in food, intolerance, or a genetically determined disease, to this enzyme, it can remove and can reduction-ize the toxicity and allergenic. In the case of a food allergy, generally, an allergen peptide has many which have high hydrophobicity. Allergenic removal and a fall are made by being changed into a hydrophilic peptide by this enzyme processing. Big effectiveness is brought about, when it especially contains glutamine residue in an allergen peptide so that the allergen of the wheat gluten origin may see.

[0059] Furthermore, by deamidating protein with this enzyme, proteinic mineral susceptibility can be reduced, the fusibility mineral content in protein and a mineral solution can be raised, and the absorptivity to the body of a mineral can be raised. Generally it is known well that it will improve if the absorptivity to the body of the calcium in food makes calcium solubilize using an organic acid or a casein phospho peptide. It is possible to make a lot of cull RUSHIMU solubilize by making protein deamidate with this enzyme according to the same mechanism. A high mineral (for example, calcium) content drink and the absorption enhancers of a mineral (for example, calcium) can also be manufactured using this deamidation protein.

[0060] Furthermore, in an amino acid system seasoning (hydrolyzate (HAP) of an animal protein, and hydrolyzate of vegetable albumen (HVP)) or bean paste, and soy sauce manufacture, effectiveness, such as a fall of bitterness, improvement in the rate of proteolysis of a protease, and enhancement of a glutamic-acid content, is brought about. Generally, it is well known that the cause of bitterness originates in a hydrophobic peptide, and reduction-ization of bitter peptides is brought about by deamidation. It is also known that the peptide which has glutamic acid in an amino terminal has the masking effect of bitterness. Moreover, by deamidation, since the primary structure of raw material protein and higher order structure change, the protease susceptibility of the protein can also be raised. In a result, HAP like an enzyme, and HVP manufacture, the low cracking severity which was one in question is also improvable. Moreover, on the other hand in HAP and HVP manufacture, the fall of the glutamic-acid content by pyroglutamic acid generation was a problem. Although intramolecular circularization of the glutamine of isolation generates this pyroglutamic acid, by deamidating raw material protein, this can be prevented and enhancement of a glutamic-acid content is brought about as a result.

[0061] Furthermore, it can be used also as a reaction control agent of transglutaminase. Transglutaminase begins the food field as proteinic modifier, i.e., enzyme for bridge formation, and is widely used for industrial use. Although aimed at raising the functionality of obtaining a proteinic gelation object by the protein crosslinking reaction of transglutaminase, or protein, it was difficult to control crosslinking reaction, such as stopping at the suitable time acquiring the product which has each application, and the degree of cross linking according to the purpose and functionality, i.e., a reaction. Especially the thing for which transglutaminase inhibitors generally known, such as

EDTA, and an ammonium chloride or an SH reagent, are added in the case of reforming of food-grade protein was not desirable.

[0062] By adding the protein deamidase by this invention during the reaction of transglutaminase at the suitable time, it is possible to stop a transglutaminase reaction. That is, a transglutaminase reaction can be stopped by changing into glutamic-acid residue the glutamine residue which is the target of the transglutaminase reaction in a protein matrix by the protein deamidase.

[0063] In this case, although it is required for compatibility with the glutamine residue in the protein which is a substrate of the protein deamidase to be higher than that of transglutaminase Since the water which exists in the reaction environmental Nakatoyo wealth other than glutamine residue in the case of the former is only needed in the latter reaction to epsilon-amino group of lysine residue other than glutamine residue being required It can presume that the direction of the reaction of the protein deamidase generally precedes with the reaction of transglutaminase. Of course, a protein matrix is beforehand processed suitably by the protein deamidase, and if the back transglutaminase reaction which changed desired glutamine residue into glutamic-acid residue is presented, the protein reforming object of a desired degree of cross linking and a protein gelation object can be obtained.

[0064] Furthermore, it can be used also as proteinic object for a functional alteration, i.e., reagent for protein engineering. When a protein matrix is an enzyme, an enzyme chemistry-[the enzyme] physicochemical quality can be changed. For example, by carrying out deamidation of the enzyme protein with this enzyme, the isoelectric point of an enzyme protein can fall and pH stability can be changed. Moreover, the affinity of substrate of the enzyme, substrate specificity, a reaction rate, pH dependency, temperature dependence, temperature stability, etc. are changeable by changing the structure and the electric environment of an active site.

[0065] Furthermore, it can be used also as a protein analysis and reagents for research, such as a proteinic reagent for amide content quanta, and a proteinic reagent for solubilization.

[0066] Furthermore, it can use for improvement in extract / concentration effectiveness of cereals and legumes protein etc. Although water generally has much insoluble protein as for the protein of cereals, such as wheat and an soybean, or legumes and it is not easy to extract protein, by processing the suspension of wheat flour or a soybean meal with this enzyme, and solubilizing protein, protein can be extracted easily and the protein isolation object of a high content can be obtained.

[0067] In case protein is generally extracted from defatted soybean powder or a flake (about 50% of protein contents) in the case of soybean protein, after making protein insolubilize first by heat treatment, ethanol processing, or isoelectric point processing of the pH4.5 neighborhood, except for the polysaccharide of fusibility, the soybean protein concentrate (concentrate) of about 70% of protein contents is obtained. When protein of a high grade is furthermore desired, a soybean meal and a concentrate are ****(ed) and dissolved at dilution alkali, protein is dissolved, and it is adjusted except for the insoluble matter. This thing is called a soybean protein isolation object (isolation), and contains protein about 90%. These soybean protein products are used for various food including a hum sausage or the food for sucklings using functionality and high nutritive values, such as the emulsifiability of soybean protein, a gelation property, and water retention.

[0068] If this enzyme is used in case these soybean protein products are manufactured, not only the improvement in yield but a more high-concentration protein product can be manufactured by soluble improvement in protein. Since it deamidates the protein product obtained by doing still in this way, it excels in functionality. Therefore, manufacture of the food which shows the effectiveness which was excellent when it was used for various food, such as meat, a fish meat product, and noodles, and has a new texture and a new function is attained.

[0069] Cultivate the transformant which introduced the recombination vector which contains hereafter the gene which carries out the code of the protein deamidase of this invention, and the protein deamidase, and this gene, and this vector, and this transformant to a culture medium, the protein deamidase is made to produce, and the manufacturing method of the protein deamidase characterized by extracting the protein deamidase from a culture is explained further.

[0070] Although all the protein deamidase obtained by the manufacturing method of the protein deamidase mentioned above as protein deamidase of this invention is contained, the polypeptide in which one piece or two or more amino acid residue have the amino acid sequence by which at least one of deletion, addition, insertion, or the permutations is made especially in an amino acid sequence given in the array number 6 of an array table is desirable, and the polypeptide which has the amino acid sequence of a publication for the array number 6 of an array table is more more desirable still.

[0071] The gene which has homology is raised to a gene acquirable [with cloning of this gene] from the microorganism which produces this protein deamidase as a gene which carries out the code of the protein deamidase of this invention, or this gene. As homology, the gene which has at least 60% or more of homology, the gene which has 80% or more of homology preferably, and the gene which has 95% or more of homology still more preferably can be raised. As a gene which carries out the code of the protein deamidase of this invention, the following nucleotides (DNA or RNA) are desirable.

[0072] The nucleotide which carries out the code of the polypeptide which has the activity which consists of a nucleotide chosen from following (a) - (g), and carries out deamidation of the amide group in protein.
(a) In an amino acid sequence given in the array number 6 of a nucleotide and the (b) array table which carries out the code of the polypeptide which has the amino acid sequence of a publication to the array number 6 which is an array table The nucleotide to which one piece or two or more amino acid residue carry out the code of the polypeptide which has the amino acid sequence by which at least one of deletion, addition, insertion, or the permutations is made, (c) In a base sequence given in the array number 5 of a nucleotide and the (d) array table which has the base sequence of a publication for the array number 5 of an array table The nucleotide in which one piece or two or more bases have the base sequence by which at least one of deletion, addition, insertion, or the permutations is made, (e) The gene hybridized under stringent conditions to a nucleotide given in either of above-mentioned (a) - (d), (f) Nucleotide of the nucleotide which has homology in a nucleotide given in either of - (d), and (above-mentioned a) (g) above-mentioned (a) - (f) which degenerates to the nucleotide of any one publication at least.
[0073] The gene which carries out the code of the protein deamidase of this invention is acquirable from the microorganism which produces the protein deamidase mentioned above by performing cloning of this gene by approach which is indicated below, for example. First, the protein deamidase of this invention is isolated and refined by the above-mentioned approach, and the information about the partial amino acid sequence is acquired from the microorganism which produces the protein deamidase.
[0074] As the partial amino acid sequence decision approach, a direct conventional method is followed in the refined protein deamidase, for example. Edman degradation method [journal OBU biological chemistry, ** By 256 volumes and 7990-7997th page (1981)], amino acid sequence analysis [protein-sequencer 476A, Applied May present], such as biotechnology systems (Applied Biosystems) company make, and Or it is effective to make proteolytic enzyme act, to perform limited hydrolysis, to carry out separation purification of the obtained peptide fragment, and to perform amino acid sequence analysis about the obtained purification peptide fragment.
[0075] In this way, cloning of the protein deamidase gene is carried out based on the information on the partial amino acid sequence acquired. Cloning can be performed using being general, the approach using PCR, or a hybridization method.
[0076] Molecular, when using a hybridization method Cloning, A Laboratory The approach of a publication can be used for a manual [Molecular Cloning, A Laboratory Manual, T. Maniatis (T.Maniatis) other work, a cold spring harbor laboratory (Cold Spring Harbor Laboratory), and the 1989 issue].
[0077] moreover The following approaches can be used when using the PCR method. First, genomic DNA of the microorganism which produces the protein deamidase It considers as mold and the synthetic oligonucleotide primer designed based on the information on a partial amino acid sequence is used. An PCR reaction is performed and the target gene fragment is obtained. PCR Law is PCR. According to the approach of a publication, it carries out to technology [PCR Technology, ERURIHHI (Erlich) HA edit, the Stockton press company (Stocktonpress), and the 1989 issue]. Furthermore, this magnification If a base sequence is determined by the approach usually used about a DNA fragment, for example, the dideoxy chain terminator method, during the determined array, the array corresponding to the partial amino acid sequence of the protein deamidase is found out in addition to the array of a synthetic oligonucleotide primer, and a part of target protein deamidase gene can be acquired. Cloning of the gene which carries out the code of the protein deamidase overall length can be carried out by performing a hybridization method etc. further by using as a probe the gene fragment obtained, of course.
[0078] At the following example 26, it is KURISEO bacterium GUREUMU. The gene which carries out the code of the protein deamidase was determined using the PCR method using JCM2410. KURISEO bacterium GUREUMU Having indicated all the base sequences of the gene which carries out the code of the protein deamidase of the JCM2410 origin for the array number 5, and indicating the amino acid sequence in which a code is carried out by this for the array number 6 was determined. In addition, although the base sequence corresponding to the amino acid sequence indicated for the array number 6 exists innumerably in addition to what was indicated for the array number 5, these are all contained in the range of this invention.
[0079] It can carry out based on the information on an amino acid sequence given in the array number 6, or a base sequence given in the array number 5, and the target gene can also be obtained by chemosynthesis (bibliography: Gene, 60 (1), and 115-127 (1987)). Moreover, the protein deamidase gene of this invention is contained in this invention as long as the polypeptide to which they carry out the code also of the gene to which one piece or two or more amino acid residue hybridize the polypeptide which has the amino acid sequence by which at least one of deletion, addition, insertion, or the permutations is made under stringent conditions in an amino acid sequence given in the array number 6 to the nucleotide and this nucleotide which carry out a code, the nucleotide which has homology in this nucleotide, and the nucleotide which degenerates to this nucleotide has protein deamidation enzyme activity.
[0080] "The bottom of a stringent condition" here says the following conditions. namely, -- 0.5%SDS and 5x DIN HARUTSU [Denhartz's, 0.1% bovine serum albumin (BSA), 0.1% polyvinyl pyrrolidone, and the 0.1% ficoll 400] --

and -- .100microg [/ml] salmon sperm DNA included 6xSSC -- in inside (1xSSC is 0.15 M NaCl, a 0.015 M sodium citrate, and pH7.0), the conditions kept warm at 50 degrees C - 65 degrees C 4 hours - overnight are said.
[0081] KURISEO bacterium GUREUMU Genomic DNA of the microorganism which produces other protein deamidase, using the whole protein deamidase gene by which all base sequences were clarified using JCM2410, or a part as a probe for hybridization The protein deamidase gene of a library or a cDNA library to the array table 5, and homology's high DNA It can sort out.
[0082] Hybridization can be performed under the stringent conditions shown above. For example, the nylon film which fixed the genomic DNA library or cDNA library obtained from the microorganism which produces the protein deamidase is created, and it is 6xSSC, 0.5%SDS, 5x DIN HARUTSU, and 100. mug/ml salmon sperm DNA The nylon film is blocked at 65 degrees C among the included pre hybridization solution. Then, each probe which carried out the label by 32P is added, and it is kept warm at 65 degrees C overnight. It is 6xSSC about this nylon film. 2xSSC which contains SDS 0.1% for 10 minutes at a room temperature inside 0.2xSSC which contains SDS 0.1% for 10 minutes at a room temperature inside DNA which takes autoradiography and is specifically hybridized with a probe after washing for 30 minutes at 45 degrees C, inside and It is detectable. Moreover, the gene which shows various homology can be obtained by changing conditions, such as washing.
[0083] On the other hand, it is from the base sequence of the gene of this invention. The primer for an PCR reaction can be designed. This primer is used. By performing an PCR reaction, the high gene fragment of the gene of this invention and homology can be detected, or that whole gene can also be obtained further.
[0084] In order to check whether it is the gene to which the obtained gene carries out the code of the polypeptide which has the target protein deamidation enzyme activity, the determined base sequence can also be presumed from the gene structure and homology as compared with the base sequence of the protein deamidase of this invention, or an amino acid sequence. Moreover, it can check whether it is the gene which carries out the code of the polypeptide which has the target protein deamidation enzyme activity by manufacturing the polypeptide of the obtained gene and measuring protein deamidation enzyme activity.
[0085] For producing the polypeptide which has protein deamidation enzyme activity using the protein deamidase gene of this invention, the following approaches are expedient. First, the polypeptide which has protein deamidation enzyme activity can be made to produce by performing a host's transformation using the vector containing the target protein deamidase gene, and subsequently performing culture of this transformant on the conditions usually used.
[0086] Moreover, a microorganism, an animal cell, a plant cell, etc. can be used as a host. As a microorganism, it is .
[0087] to which the network of a baculovirus is mentioned as a . animal cell to which mold, such as yeast, such as bacteria, such as Escherichia coli, a Bacillus group, a Streptomyces group, and a Lactococcus group, a Saccharomyces group, a Pichia group, and a Kluyveromyces group, an Aspergillus group, a Penicillium group, and a Trichoderma group, is mentioned. Although it is simple to perform the check of a manifestation and the check of a manifestation product using the antibody to the protein deamidase, a manifestation can also be checked by measuring protein deamidation enzyme activity.
[0088] For refining the protein deamidase, it can carry out as mentioned above from the culture of a transformant, combining suitably various chromatographies, such as centrifugal separation, UF concentration, a salting-out, and ion exchange resin.
[0089] Moreover, when the primary structure and gene structure of the protein deamidase became clear by this invention, one piece or two or more amino acid residue are able to introduce random variation or site-specific mutation, and to obtain the gene with which at least one of deletion, addition, insertion, or the permutations is made in the amino acid sequence of the natural protein deamidase using the gene of this invention. Thereby, although it has protein deamidation enzyme activity, it becomes it is possible to obtain the gene which carries out the code of the protein deamidase from which properties, such as optimum temperature, stable temperature, optimal pH, Stability pH, and substrate specificity, differed for a while, and possible to manufacture these protein deamidase in gene engineering.
[0090] As an approach of introducing random variation, for example as an approach of processing DNA chemically Approach [pro C DINGUZU OBU THE National which makes the transition variation which a sodium hydrogensulfite is made to act and changes a cytosine base into a uracil base cause Academy OBU Sciences OBU THE USA, the 79th volume, As the 1408-1412page(1982)] and a biochemical process, under [alpha-S] dNTP existence, The approach [gene (Gene) which produces base exchange in the process which compounds a double strand, the 64th volume, and the 313-319th as an approach using page (1988)] and PCR Manganese is added to the system of reaction. Approach [analytical biochemistry which performs PCR and makes correctness of the incorporation of a nucleotide low (Analytical Biochemistry), The 224th A volume and the 347-353rd Page (1995)] etc. can be used.
[0091] the approach [GYAPPUDO duplex (gapped duplex) which uses amber mutation as an approach of introducing site-specific mutation, for example -- law -- NUKUREIKKU ASHIZZU Research (Nucleic Acids Research), the approach of using the recognition site of the 12th volume, No. 24, the 9441-9456page(1984)], and a restriction enzyme -- [-- analytical biochemistry -- ** 200 volumes, the 81-88th page (1992), a gene, ** 102 volumes, the 67-70page

(1991)], and dut (dUTPase) and approach [Kunkel (Kunkel) using ung variation (uracil DNA glycosylase) -- law -- Pro C DINGUZU OBU THE National OBU Sciences OBU THE USA, The 82nd volume and the 488-492nd Page (1985)], DNA polymerase -- and -- the approach [oligonucleotide-DAIREKU Ted dual umber (Oligonucleotide-directed Dual Amber:ODA) using the amber mutation using a DNA ligase -- law and a gene -- ** 152 volumes and the 271-275th A page (1995), JP,7-289262,A], How (publication-number 8 No. -70874 official report) to use the host who made the restoration system of DNA guide, How (JP,8-140685,A) to use the protein which carries out the catalyst of the DNA strand exchange reaction, Two kinds of primers for variation installation which added the recognition site of a restriction enzyme were used. The approach by PCR (USP5,512,463), Double strand which has an inactivation drug resistance gene A DNA vector and two kinds of primers were used. The approach [gene by PCR, ** 103 volumes, the 73-77page(1991)], and amber mutation were used. The approach [the international public presentation WO 98/No. 02535 official report] by PCR etc. can be used.

[0092] Moreover, site-specific mutation can be easily introduced by using the kit marketed. As a commercial kit, it is GYAPPUDO, for example. The duplex method was used. Mutan(trademark)-G (TAKARA SHUZO CO., LTD. make), The Kunkel method was used. Mutan(trademark)-K (TAKARA SHUZO CO., LTD. make), ODA Mutan-(trademark) Express Km (TAKARA SHUZO CO., LTD. make) using law, The primer for variation installation, and PIROKOKKASU Julio Sas (Pyrococcus furiosus) origin QuikChangeTM Site-Directed Mutagenesis using DNA polymerase Kit [the Stratagene (STRATAGENE) make] etc. -- it can use -- moreover As a kit using the PCR method TaKaRa LA PCR in vitro Mutagenesis Kit (TAKARA SHUZO CO., LTD. make), Mutan-(trademark) Super Express Km, etc. can be used (TAKARA SHUZO CO., LTD. make).

[0093] Thus, gene engineering-manufacture [that the polypeptide which has protein deamidation enzyme activity is cheap, and high grade] is attained by having offered the primary structure and gene structure of the protein deamidase by this invention. In addition, although various reference etc. was quoted on these specifications, these are all included in this specification as reference.

[0094] Hereafter, although this invention is explained in full detail using an example, this invention is not limited to these. In the limitation and this specification which are not specified, W/V % showed % the following.

[0095]

[Example] By the lactose culture medium which mentioned above example 1 KURISEO bacterium GUREUMU (Chryseobacterium gleum) JCM2410, shaking culture of the 30 degrees C was carried out for six days. The culture progress is shown in drawing 1 .

[0096] The culture progress about KURISEO bacterium indolo GENESU (Chryseobacterium indologenes) IFO14944, the KURISEO bacterium MENINGOSE petit cam (Chryseobacterium meningosepticum) IFO12535, and KURISEO bacterium ballast CHINAMU (Chryseobacterium balustinum) IFO15053 is shown in drawing 6 -8 like example 2 example 1.

[0097] It cultivated about Flavobacterium AKUATIRE (Flavobacterium aquatile) IFO15052 like example 3 example 1. The protein deamidation enzyme activity in culture medium is shown in Table 3.

[0098] It is en PEDOBAKUTA like example 4 example 1. It cultivated about brevis (Empedobacter brevis) IFO14943. The protein deamidation enzyme activity in culture medium is shown in Table 3.

[0099] It cultivated like example 5 example 1 about Sphingobacterium SUPIRICHIBORAMU (Sphingobacterium spiritivorum) IFO14948 and Sphingobacterium HEPARINAMU (Sphingobacterium heparinum) IFO12017. The protein deamidation enzyme activity in culture medium is shown in Table 3.

[0100] It cultivated like example 6 example 1 about the AUREO bacterium ASUTERO aroma tee cam (Aureobacterium esteraromatidum) IFO3751. The protein deamidation enzyme activity in culture medium is shown in Table 3.

[0101] It cultivated about MIROIDESU ODORATASU (Myroides odoratus) IFO14945 like example 7 example 1.

[0102] which shows the protein deamidation enzyme activity in culture medium in Table 3

[Table 3]

菌　株	培養時間(h)	脱アミド活性 (U/ml)	
		Z-Gln-Gly	カゼイン
Flavobacterium aquatile IFO15052	48	0.019	0.038
Empedobacter brevis IFO14943	20	0.040	0.149
Sphingobacterium spiritivorum IFO14948	20	0.057	0.078
Sphingobacterium heparinum IFO12017	48	0.047	0.031
Aureobacterium esteroaromaticum IFO3751	31	0.003	0.019
Myroides odoratus IFO14945	41	0.005	0.026

EXAMPLE

[Example] By the lactose culture medium which mentioned above example 1 KURISEO bacterium GUREUMU (Chryseobacterium gleum) JCM2410, shaking culture of the 30 degrees C was carried out for six days. The culture progress is shown in drawing 1 .
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[0101] It cultivated about MIROIDESU ODORATASU (Myroides odoratus) IFO14945 like example 7 example 1.
[0102] which shows the protein deamidation enzyme activity in culture medium in Table 3
[Table 3]

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Myroides odoratus IFO14945	41	0.005	0.026

[0103] Production of the protein deamidase was checked by any strain in examples 1-7.
[0104] The yeast extract with which production of the protein deamidase was checked by any strain used for examples 1-5 when it replaced with the example 8 lactose culture medium and cultivated similarly using the following culture media 0.25% poly peptone 0.3% casein sodium 0.25%Na2HPO4.12H2O 0.3%MgSO4 and 7H2O 0.02 % (pH7.0)
[0105] It is 2.0M after condensing about 17 times by the ultrafiltration (UF) film (SEP-0013, Asahi Chemical make) about the centrifugal supernatant liquid which was obtained in the example 9 example 1 and which was removed and obtained [fungus body] in 24-hour culture medium according to 4 degrees C, 12000 rpm (22200xg), and the centrifugal separation for 15 minutes. It dialyzed at 4 degrees C overnight to 10mM sodium phosphate buffer solution (pH6.5) containing NaCl. Phenyl sepharose which equilibrated the obtained centrifugal supernatant liquid with 10mM sodium phosphate buffer solution (pH6.5) containing 2.0M NaCl after removing the produced precipitate according to 4 degrees C, 10000 rpm (12300xg), and the centrifugal separation for 15 minutes The CL-6B column (Pharmacia manufacture) was presented and the protein to which it stuck by the NaCl linear density gradient of 2.0M to 0M was made to elute.

[0106] Protein deamidation activity fractions were collected, S-sephacryl 100 column which equilibrated with 10mM sodium phosphate buffer solution (pH6.5) which contains 0.6M NaCl and 0.05%Tween 20 after concentration by ultrafiltration membrane was presented, and it eluted with this buffer solution. Protein deamidation activity fractions were collected, it dialyzed to distilled water after concentration by ultrafiltration membrane, and the protein deamidase solution was obtained.
[0107] The pattern which presented drawing 9 with the sample of each step in a purification process 10 to 20% at SDS polyacrylamide gel electrophoresis is shown. Thus, this purification enzyme preparation (lane 5) In SDS polyacrylamide gel electrophoresis, it turns out that it is the single protein of molecular weight 20kDa.
[0108] When activity was measured by the above-mentioned measuring method (the approach of making Z-Gln-Gly a substrate, and the approach of making casein a substrate), the enzyme preparation of 18.8 unit / ml (it is a substrate about Z-Gln-Gly), and 14.0 units / ml (it is a substrate about casein) was obtained. Moreover, transglutaminase activity and protease activity were not detected. Therefore, it turns out that the protease activity in the culture medium in drawing 1 is completely removed at the above-mentioned purification process. Moreover, the glutaminase activity which acts on the glutamine of isolation was not detected by this enzyme preparation, either.
[0109] The culture medium obtained in the examples 2-7 was refined like example 10 example 9. Consequently, activity as respectively shown in Table 4 was acquired. Transglutaminase activity and protease activity were not detected by the obtained protein deamidase. Therefore, it turns out that the protease activity in culture medium is completely removed at the above-mentioned purification process.
[0110]
[Table 4]

菌　株	脱アミド活性 (U/ml)	
	Z-Gln-Gly	カゼイン
Chryseobacterium indologenes IF014944	14.2	10.5
Chryseobacterium meningosepticum IF012535	13.2	10.3
Chryseobacterium balustinum IF015053	9.88	7.48
Flavobacterium aquatile IF015052	3.97	3.09
Empedobacter brevis IF014943	2.11	1.48
Sphingobacterium spiritivorum IF014948	1.59	1.25
Sphingobacterium heparinum IF012017	7.43	5.20
Aureobacterium esteroaromaticum IF03751	1.44	1.15
Myroides odoratus IF014945	3.06	2.36

[0111] Example 11 They are 100ml 176mM(s) in 1g of preparation wheat gluten of deamidation gluten. It ****(ed) to the sodium phosphate buffer solution (pH 6.5), the protein deamidase of 5U was added, and the shaking reaction was carried out at 37 degrees C for 20 hours. The ammonia at this time or the isolation pattern of ammonium is shown in drawing 10 . Thus, it turns out that ammonia separated and the deamidation reaction has arisen with advance of reaction time in the reaction of enzyme addition to the enzyme additive-free reaction performed as contrast. After the reaction, it dialyzed to distilled water, and it freeze-dried and deamidation gluten powder was obtained.
[0112] The rate of deamidation of the obtained deamidation gluten was 37.4%. In addition, the rate of deamidation carried out the quantum of the ammonia which separated after reaction termination, and expressed it as a percentage to the total amide content of wheat gluten. The proteinic total amide content hydrolyzed protein (1%w/v) at 100 degrees C among 2-N hydrochloric acid for 2 hours, carried out the quantum of the ammonia which separated, and asked for it.
[0113] Example 12 ****, it was made to dissolve in 40mM Britton-Robinson buffer solution from pH3 of 1.0ml to 12, and 2.0mg of enzyme unsettled gluten powder obtained by the deamidation gluten powder and control experiment which were obtained in the improvement example 11 of the functionality (solubility, dispersibility) of deamidation gluten was gently put on the room temperature for 30 more minutes after the shaking for 30 minutes at the room temperature. the protein content in the supernatant liquid obtained here by taking after measuring pH and taking centrifugal separation at the low speed of 3000rpm (760xg) for 10 minutes by 24 degrees C -- BCA -- it measured by law and the content of the protein in supernatant liquid was made into the index of dispersibility (Methods of Testing Protein Functinality, p25, edited by G.M.Hall, Blackie Academic & Professional, London, 1996).
[0114] the supernatant liquid obtained by furthermore carrying out centrifugal separation of this supernatant liquid at

the high speed of 14000rpm (16000xg) for 30 minutes by 24 degrees C -- the 0.45-micrometer film -- filtering -- the protein content in filtrate -- BCA -- it measured by law. The protein content in this filtrate was made into the soluble index (Methods of Testing Protein Functinality, p47-55, edited by G.M.Hall, Blackie Academic & Professional, London, 1996).
[0115] As shown in a result, drawing 11, and drawing 12, it turns out that dispersibility and solubility of deamidation gluten are improving remarkably in the large area from the pH4.2 neighborhood to the pH12 neighborhood compared with enzyme unsettled gluten.
[0116] Example 13 The 5g deamidation gluten and the polysorbates [of 2g corn syrup 2g and 0.4g / 60 and 51g] water which were manufactured according to the manufacture example 11 of the coffee whitener using deamidation gluten were mixed, and after heating at 40 degrees C, the dipotassium phosphate of 0.3 g was added and it heated to 80 degrees C. After adding the partial hydrogenation coconut oil [of a melting condition / 6g and 0.2g] monoglyceride into this mixed liquor and leaving it for 20 minutes at 80 degrees C into it, the coffee whitener which homogenizes, cools by 211kg/cm2 and contains deamidation gluten was manufactured. This product showed good distribution and solubility, and taste, when a stable emulsion condition was shown and it added in coffee.
[0117] example 14 100g of manufacture wheat flour of tempura powder is ****(ed) in the water of 1L -- the protein deamidase of ten units was added and the wheat flour which dehydrated according to centrifugal separation after 20-hour shaking reaction processing, carried out heat desiccation, and carried out deamidation processing at 37 degrees C was obtained. 50g of this wheat flour was melted by 60ml of water, and it considered as the batter for tempura. Dusting powder was made the Taisho shrimp (about 25g), this batter was attached and fly was carried out for 3 minutes from 170-180-degree C the rapeseed / soybean preparation oil. Moreover, fly was similarly carried out using the wheat flour which does not perform protein deamidase processing. When the wheat flour which performed protein deamidase processing adjusted batter, the dispersibility was excellent and very excellent in mouthfeel, such as stiffness of the clothes of the obtained fly, and SAKUMI of clothes, an appearance, and flavor.
[0118] Example 15 The premix for hot cakes of the following presentations was prepared using the protein deamidase processing wheat flour adjusted according to the manufacture example 14 of a premix.
Protein deamidase processing wheat flour 72.0% sugar 20.0% ** agent (sodium hydrogencarbonate) 1.5% fats and oils 3.0% salt 1.0% glucono delta lactone 2.0% perfume premix 200g of the 0.5% above -- a ball -- putting in -- this -- 150ml of cow's milk, and 50g of whole eggs -- adding -- churning mixing -- carrying out -- the object for hot cakes -- the ground was prepared. It prepared similarly using unsettled wheat flour to contrast. Circularly [100g of acquired grounds] on a 160-degree C hot plate, the flesh side was calcinated for the sink and the table for 2 minutes for 4 minutes, and the hot cake was prepared.
[0119] handling of the ground, the feeling of software of a hot cake, and a feeling of ****** -- admiration was gently judged by the panelist. Consequently, when protein deamidase processing wheat flour was used, compared with contrast, it excelled in all points.
[0120] Example 16 One loaf bread was manufactured by the time in method by the combination shown below in preparation of make baker's dough.
[Combination]
Wheat flour 100 % 2000 g Sugar 5 % 100 g Salt 2 % 40 g Shortening 4 % 80 g Yeast 3 % 60 g Ascorbic acid 20 ppm40 mg Water 69 % 1380 ml [0121] The protein deamidase addition (7.5 unit / 1kg of wheat flour) group was prepared and compared with the enzyme additive-free (contrast) group on the basis of the above-mentioned combination.
[0122] [Process]
(1) Mixing : Low-speed 4 minutes -> Four high-speed minutes -> Shortening addition -> low-speed 1 minute -> Medium-speed 4 minutes -> High-speed 4-minute ** [(2)] top temperature : 27-29-degree-C(3) floor time : [27 degrees C, 30 minute (4) division] : -- the ground -- weight 450 g(5) bench time: -- 30-minute (6) HOIRO : 38 degrees C and pan case mold top 3.5 cm (7) baking : 230 degrees C and 25 minutes [0123] Handling [of the ground] became good [a protein deamidase addition group], and effectiveness was looked at by improvement in the extensibility of the ground, and improvement in SOFUTONESU of a baking pan.
[0124] example 17 the protein deamidase processing wheat flour adjusted according to the preparation example 14 of biscuit dough -- using -- the following presentations -- the object for biscuits -- the ground was prepared.
[0125]
Protein deamidase processing wheat flour 100g shortening 16g sugar 50g sodium bicarbonate 0.81g potassium bitartrate 0.5g water 16g egg 2g [0126] Mixing, shaping, and baking (180-220 degrees C) were performed according to the conventional method, and the hard biscuit was prepared. Handling of the ground and improvement in an extensibility are remarkable, and mouthfeel of the obtained biscuit was also excellent.
[0127] Example 18 Preparation milk casein 1g of deamidation casein was ****(ed) to the 100ml 176mM sodium phosphate buffer solution (pH 6.5), the protein deamidase of five units was added, and the shaking reaction was carried

out at 37 degrees C for 20 hours. The ammonia at this time or the isolation pattern of ammonium is shown in drawing 13. Thus, it turns out that ammonia separated and the deamidation reaction has arisen with advance of reaction time in the reaction of enzyme addition to the enzyme additive-free reaction performed as contrast. After the reaction, it dialyzed to distilled water, and it freeze-dried and deamidation casein powder was obtained.

[0128] The rate of deamidation of the obtained deamidation casein was 40.9%. In addition, the rate of deamidation carried out the quantum of the ammonia or ammonium which separated in the solution after reaction termination, and expressed it as a percentage to the total amide content of casein. The proteinic total amide content hydrolyzed protein (1%w/v) at 100 degrees C among 2-N hydrochloric acid for 2 hours, carried out the quantum of the ammonia which separated, and asked for it. Moreover, the pattern which presented drawing 14 with this deamidation casein 10 to 20% with enzyme unsettled casein at SDS polyacrylamide gel electrophoresis is shown. deamidation casein (lane 2) -- it turns out that the molecular weight is not changing, i.e., have not produced decomposition or crosslinked polymerization, either. Since the mobility in electrophoresis decreased as a result of becoming small compared with the casein which association with SDS which has the same negative charge since the proteinic negative charge increased this by deamidation, although it was observed that the band of deamidation casein has shifted to a macromolecule side slightly here decreases according to electrostatic repulsion, and does not deamidate the whole negative charge as a result, it thinks.

[0129] Example 19 It ****, 2.0mg of enzyme unsettled casein powder obtained by the deamidation casein powder and control experiment which were obtained in the improvement example 18 of calcium solubility of deamidation casein was dissolved in 10mM tris hydrochloric-acid buffer solution (pH7.1) containing the calcium chloride of 0-30mM, and it put on the room temperature gently for 30 more minutes after the shaking for 30 minutes at the room temperature. the supernatant liquid obtained by carrying out centrifugal separation of this liquid at the low speed of 3000rpm (760xg) for 10 minutes by 24 degrees C, and carrying out centrifugal separation of the supernatant liquid at the high speed of 14000rpm (16000xg) for 30 minutes by 24 more degrees C -- the 0.45-micrometer film -- filtering -- the protein content in filtrate -- BCA -- it measured by law. The protein content in this filtrate was made into the index of calcium solubility.

[0130] a result -- drawing 15 -- being shown -- as -- deamidation casein -- enzyme unsettled casein -- comparing -- the bottom of high concentration calcium existence -- also setting -- high solubility -- being shown -- remarkable -- calcium solubility -- improving -- **** -- things -- understanding .

[0131] Example 20 150ml of brine was added to manufacture gluten 45g of zymolysis seasoning liquid 15%, protein deamidase 50 unit, glutaminase F100 (product made from Amano Pharmaceuticals) 0.1%, and protease M(product made from Amano Pharmaceuticals)0.75% were added, and it reacted for three - four days at 45 degrees C. It heated for 20 minutes at 90 degrees C after the reaction, and zymolysis seasoning liquid was prepared. As compared with the seasoning liquid which operated similarly and was prepared without adding the protein deamidase as contrast, both cracking severity and catabolic rate were able to improve, and were able to manufacture seasoning liquid with it. [there is little generation of the bitterness component of the obtained seasoning liquid, and good]

[0132] Example 21 pH was adjusted to 6.5, having carried out **** and the dissolution of the 100g of the manufacture soybean meals of deamidation soybean protein, and stirring them in the water of 1L, at the concentration / recovery approach list of soybean protein, the protein deamidase of 500 units was added, and stirring was continued at the room temperature for 2 hours. The reaction back pH was adjusted to 8 and discard was removed at the room temperature after 1-hour stirring according to 10000rpm (12300xg) and the centrifugal separation for 30 minutes. In order to collect protein from the obtained supernatant liquid, after performing heat treatment for 30 minutes at 80 degrees C, 10000rpm (12300xg) and the precipitate produced according to the centrifugal separation for 30 minutes were collected, it was made to dry and protein powder was obtained. This protein preparation had 95% of protein content, and its yield from a soybean meal was also as high as about 40%.

[0133] Example 22 Coagulation processing is performed by repeating churning, extrusion, and rolling and performing deamidation soybean protein manufactured according to the manufacture example 21 of the sausage using deamidation soybean protein. After kneading the congelation, the raw material meat, and the various spices of soybean protein which were obtained to the following combination, casing was filled up according to the conventional method and the sausage was prepared. The obtained product was very excellent in mouthfeel.

[0134]

Buta -- shoulder butt -- 500g cow round 500g Buta ****** 100g congelation 100g salt 25g niter 3g sugar 5g Ajinomoto 3g White pepper 3g nutmeg 4g cinnamon 0.5g onion juice 5g [0135] Example 23 12.5micro of solutions l which contain the transglutaminase (it prepares according to Agric.Biol.Chem., 53 No. 10, 2613 - 2617 pages, and 1989) of the Streptoverticillium origin of 0.0125 units in 25micro (pH 7.0) of 20mM phosphate buffers l containing 10% casein of use as a transglutaminase reaction control agent was added, and the standing reaction was carried out at 37 degrees C after stirring. 1 hour after, 12.5micro of solutions l containing the protein deamidase of 0.0188 units was added, and it put on 37 more degrees C gently after stirring. A part of reaction mixture was isolated preparatively

during a reaction and 1, 2, and 4 or 24 hours after, and the SDS-polyacrylamide gel electrophoresis using SDS-polyacrylamide gel was presented 2 to 15%. What added water instead of the protein deamidase solution as a control experiment was performed.
[0136] The result is shown in drawing 16 and the sample of each lane of drawing 16 is shown in the following table 5.
[0137]
[Table 5]

レーン	蛋白質脱アミド酵素	時間 (h)
1	−	0
2	−	1
3	−	2
4	−	4
5	−	24
6	−	0
7	+	1
8	+	2
9	+	4
10	+	24

[0138] a result -- drawing 16 -- like -- the protein deamidase -- having not added -- contrast -- at a reaction, it turns out that after addition is not changing from the pattern at the addition time (lane 7) by the reaction which added the protein deamidase to the band of a casein monomer which giant-molecule-ization by proteinic crosslinking reaction was observed with the passage of time, and was observed in 0 hour decreasing in number and disappearing. This means that the crosslinked polymer-ized reaction of transglutaminase stopped by addition of the protein deamidase.
[0139] 12.5micro of solutions l which contain the transglutaminase of the Streptoverticillium origin of 0.0125 units in 25micro (pH7.0) of 20mM phosphate buffers l containing deamidation casein 10% obtained in the example 18 was added, and the standing reaction was carried out at 37 degrees C after stirring. A part of reaction mixture was isolated preparatively during a reaction and 1, 2, and 4 or 24 hours after, and the SDS-polyacrylamide gel electrophoresis using 2 - 15%SDS-polyacrylamide gel was presented. The thing using unsettled casein was also performed instead of deamidation casein as a control experiment. The result is shown in drawing 17 and the sample of each lane of drawing 17 is shown in the following table 6.
[Table 6]

レーン	基質蛋白質	時間 (h)
1	脱アミド化カゼイン	0
2	脱アミド化カゼイン	1
3	脱アミド化カゼイン	2
4	脱アミド化カゼイン	4
5	脱アミド化カゼイン	24
6	カゼイン	0
7	カゼイン	1
8	カゼイン	2
9	カゼイン	4
10	カゼイン	24

a result -- drawing 17 -- like -- enzyme unsettled casein -- having used -- contrast -- at a reaction, it turns out by the reaction using deamidation casein that after a reaction is not changing from the band (lane 1) of 0 hour to the band of a casein monomer which giant-molecule-ization by proteinic crosslinking reaction was observed with the passage of time, and was observed in 0 hour decreasing in number and disappearing. It is shown that the protein with which the protein deamidase deamidated this cannot turn into a substrate of transglutaminase.
[0140] Example 24 The manufacture marketing cow's milk of the pudding Mr. food by transglutaminase and the protein deamidase was condensed with vacuum concentration, the addition dissolution of the 5g of the sugar was carried out at 100ml of this concentration liquid, and the transglutaminase of the Streptoverticillium origin was incubated at 1 unit **** and 55 degrees C. It cooled, after carrying out 1.5 unit **** stirring of the protein deamidase and stopping a transglutaminase reaction, when suitable gel was generated. a result -- desirable softness -- pudding Mr. food was able to be manufactured.
[0141] Using the enzyme unsettled casein powder obtained by the deamidation casein powder and control experiment which were obtained in the improvement example 18 of the functionality (solubility, resolvability) of example 25 deamidation casein, the same actuation as an example 12 was performed, and the solubility of deamidation casein and part acidity were investigated. a result -- drawing 18 -- being shown -- as -- deamidation -- casein -- an enzyme -- unsettled -- casein -- comparing -- especially -- usual -- food -- pH -- a region -- it is -- pH -- four -- the neighborhood --

from -- five -- the neighborhoods -- setting -- dispersibility -- solubility -- remarkable -- improving -- **** -- things -- understanding .
[0142] An example is used for below and this invention is explained further. In addition, in this specification, especially the genetic manipulation technique was performed according to the compendium (for example, "Molecular Cloning" 2nd ed., Cold Spring Harbor Laboratoty Press, 1989), unless it indicated.
[0143] Example 26 Isolation "Current Protocols in Molecular Biology" of the isolation a chromosome DNA of the gene which carries out the code of the protein deamidase of the KURISEO bacterium GUREUMU (Chryseobacterium gleum) JCM2410 origin, and Unit 2.4 (John Wiley & Sons, Inc., 1994) are followed, and it is 100ml. 3.3ml of chromosomes DNA of 190microg [/ml] concentration was obtained from the culture.
[0144] b) The amino acid sequence analyzer (Applied Biosystems) was presented with the purification preparation of the protein deamidase obtained in the decision example 9 of a partial amino acid sequence, and the N-terminal-amino-acid array of 20 residue shown in the array number 1 was determined. Next, after returning and alkylating the purification preparation of the protein deamidase obtained in the example 9 with a performic acid, decomposition by the trypsin was performed. Reversed phase liquid chromatography was presented with the acquired decomposition product, the amino acid sequence analyzer was presented with one of the separated peptide fractions, and the internal amino acid sequence of 20 residue shown in the array number 2 was determined.
[0145] Array Number 1: Ala-Val-Ser-Val-Ile-Pro-Asp-Leu-Ala-Thr-Leu-Asn-Ser-Leu-Phe-Thr-Gln-Ile-Lys-Asn array number 2:Ser-Pro-Ser-Gly-Ser-Leu-Leu-Tyr-Asp-Asn-Asn-Tyr-Val-Asn-Thr-Asn-Cys-Val-Leu-Asn[0146] c) Based on the creation N-terminal-region amino acid sequence and internal amino acid sequence of a DNA probe by PCR, two sorts of following mixed oligonucleotides were compounded with the DNA synthesis machine (Applied Biosystems), and it considered as the PCR primer.
[0147] array number 3 sense primer: -- 5 -- '-(TA) (CG) IGTIAT(TCA) CCIGA(TC) (CT) T(TCAG)AC-3' -- array number 4 antisense primer: -- 5' - A(AG) (TCAG) AC(AG) CA(AG) TT(TCAG) GT(AG) TT(TCAG) AC-3' [0148] The PCR reaction was performed under the following conditions using Omnigene Thermal Cycler (Hybaid) by using the chromosome DNA of these primers and KURISEO bacterium GUREUMU (Chryseobacterium gleum) JCM2410 as mold.
the <PCR reaction mixture> 10 x PCR reaction buffer solution (Perkin Elmer) 5.0 muldNTP mixed liquor (respectively 2.5 mM and Promega) 4.0 mul20microM Sense primer 10.0 mul20microM antisense primer 10.0 mul distilled water 20.25microl chromosome DNA solution (190microg/(ml)) 0.5 mulTaq DNA polymerase (Perkin Elmer) 0.25microl<PCR reaction condition > stage 1: Denaturation (94 degrees C, 5 minutes) 1 cycle stage 2: Denaturation (94 degrees C, 1 minute) 30 cycles Annealing (44 degrees C, 1 minute)
Expanding (72 degrees C, 1 minute)
Stage 3: 1 cycle expanding (72 degrees C, 10 minutes) [0149] When the base sequence was checked for the DNA fragment of about 0.48 obtained kbs after cloning to pCRII (Invitrogene), the base sequence which carries out the code of the above-mentioned partial amino acid sequence just before an antisense primer immediately after a sense primer was found out. This DNA fragment was made into the DNA probe for overall-length gene cloning.
[0150] d) Probe DNA and the single band of about 3.7 kb to hybridize were checked in the Eco RI decomposition product as a result of the Southern hybridization analysis of the chromosome DNA of creation KURISEO bacterium GUREUMU (Chryseobacterium gleum) JCM2410 of a gene library. EcoRI of these about 3.7 kbs In order to carry out cloning of the DNA fragment, the gene library was created as follows. lambdaZAPII which decomposed the chromosome DNA prepared by Above a by EcoRI, and carried out EcoRI processing of the acquired decomposition product Ligation was carried out to the vector (Stratagene), packaging was carried out using Gigapack IIIgold (Stratagene), and the gene library was obtained.
[0151] e) The label of the DNA fragment of 0.48 kb obtained by the screening above c of a gene library was carried out using Megaprime DNA Labeling system (Amersham) and 32 P-alpha-dCTP. The gene library obtained by d was screened by the plaque hybridization by making this into a DNA probe. After collecting phage from the obtained electropositive plaque, according to the instruction of Stratagene, plasmid p7T-1 containing an about 3.7 kb Eco RI fragment was obtained by the in vivo Exicision method.
[0152] f) the base sequence of decision plasmid p7T-1 of a base sequence -- a law -- it determined according to the method. The base sequence which carries out the code of the protein deamidase is shown in the array number 5. Moreover, the amino acid sequence in which a code is carried out by the array number 5 is shown in the array number 6. The N-terminal-region amino acid sequence (array number 1) and internal amino acid sequence (array number 2) which were determined by b in this amino acid sequence were found out.
[0153]
Array number 5GCAGTCAGTGTTATTCCTGATCTGGCAACGCTGAACAGTTTATTTACCCAG
ATCAAAAACCAGGCTTGCGGAACTTCTACAGCATCTTCTCCTTGTATCACC
TTCAGATATCCGGTTGACGGATGTTATGCAAGGGCTCACAAAATGAGACAA

ATCCTATTGAACGCCGGCTATGACTGTGAAAAGCAGTTCGTATATGGTAAT
CTGAGAGCTTCTACAGGAACATGCTGTGTATCATGGGTATATCACGTAGCA
ATTTTGGTAAGCTTCAAAAATGCTTCAGGAATTGTTGAAAAAAGAATCATA
GATCCTTCATTATTCTCCAGCGGTCCTGTAACAGATTCTGCATGGAGAGCT
GCATGTACCAACACAAGCTGCGGATCTGCGTCTGTATCTTCCTACGCCAAT
ACAGCAGGAAATGTTTACTACAGAAGTCCGTCAGGTTCATTACTGTATGAT
AACAACTATGTGAATACCAATTGTGTATTAAACATATTCTCATCCCTTTCA
GGATGTTCTCCTTCCCCAGCACCAAGTGTAGCAAGCTGTGGATTT (555 bp) [0154] Array number
6AVSVIPDLATLNSLFTQIKNQACGTSTASSPCITFRYPVDGCYARAHKMRQILLNAGYDCEKQFVYGNLRAST
(185 amino acids) [0155] The open reading frame of this gene is shown in the array number 11. As shown in the array number 12, the code of the whole is carried out as a Prepro object of 319 amino acid residue, and a Prepro field and the 185 remaining residue correspond [134 residue (underline section of the following array number 11) of N-end] to a mature object inside (see the array number 6). Since 21 residue of N-end has the description of a signal sequence among Prepro field 134 residue, a Pre field is presumed, and the 113 remaining residue is presumed to be a Pro field. This invention is not limited to especially the nucleotide that carries out the code of the polypeptide which has protein deamidation activity, or it, and includes the nucleotide which carries out the code of the still longer polypeptide which consists of a polypeptide which has protein deamidation activity, or it (for example, a Prepro object, a Pro object, etc.). [0156]

array number 11

AATAAGTGAACTATTACAATTAAAAAGTTCACTAAAACTAAACACCAAAATATAAAAACT
ATGAAAAAATTTCTGTTATCCATGATGGCATTCGTGACGATTCTGTCATTCAATGCCTGC 1 M K K F L L S
M M A F V T I L S F N A C 20
TCAGATTCAAGTGCCAACCAGGACCCGAATCTTGTCGCTAAAGAATCTAACGAAGTCGCT 21 S D S S A N
Q D P N L V A K E S N E V A 40
ATGAAAGATTTCGGTAAGACTGTTCCGGTAGGGATTGAAAAAGAAGATGGAAAATTTAAA 41 M K D F G
K T V P V G I E K E D G K F K 60
ATCTCATTTATGGTTACTGCCCAGCCGTATGAAATTGCGGACAGTAAAGAAAATGCAGGT 61 I S F M V T
A Q P Y E I A D S K E N A G 80
TATATTTCCATGATCAGACAGGCTGTTGAGAATGAAACTCCCGTTCATGTTTTCCTTAAA 81 Y I S M I R Q
A V E N E T P V H V F L K 100
GTCAACACCAATAAAATTGCAAAAGTAGAAAAAGCAACAGATGATGACATCCGTTATTTT 101 V N T N K
I A K V E K A T D D D I R Y F 120
AAATCTGTATTCAACAAGCAAGAGAGAGGTGAAAGCAACAAAGCAGTCAGTGTTATTCCT 121 K S V F N
K Q E R G E S N K A V S V I P 140
GATCTGGCAACGCTGAACAGTTTATTTACCCAGATCAAAAACCAGGCTTGCGGAACTTCT 141 D L A T L N
S L F T Q I K N Q A C G T S 160
ACAGCATCTTCTCCTTGTATCACCTTCAGATATCCGGTTGACGGATGTTATGCAAGGGCT 161 T A S S P C I
T F R Y P V D G C Y A R A 180
CACAAAATGAGACAAATCCTATTGAACGCCGGCTATGACTGTGAAAAGCAGTTCGTATAT 181 H K M R Q
I L L N A G Y D C E K Q F V Y 200
GGTAATCTGAGAGCTTCTACAGGAACATGCTGTGTATCATGGGTATATCACGTAGCAATT 201 G N L R A S
T G T C C V S W V Y H V A I 220
TTGGTAAGCTTCAAAAATGCTTCAGGAATTGTTGAAAAAAGAATCATAGATCCTTCATTA 221 L V S F K N
A S G I V E K R I I D P S L 240
TTCTCCAGCGGTCCTGTAACAGATTCTGCATGGAGAGCTGCATGTACCAACACAAGCTGC 241 F S S G P V
T D S A W R A A C T N T S C 260
GGATCTGCGTCTGTATCTTCCTACGCCAATACAGCAGGAAATGTTTACTACAGAAGTCCG 261 G S A S V S
S Y A N T A G N V Y Y R S P 280
TCAGGTTCATTACTGTATGATAACAACTATGTGAATACCAATTGTGTATTAAACATATTC 281 S G S L L Y
D N N Y V N T N C V L N I F 300
TCATCCCTTTCAGGATGTTCTCCTTCCCCAGCACCAAGTGTAGCAAGCTGTGGATTTTAA 301 S S L S G C S
P S P A P S V A S C G F * 319
TTTTGATACATTGCAGGAGCTTTTTATTTAATACTTTTTATTATGAAAGCCTGGTCCTAT [(1080)0157]
Array number 12M K K F L L S M M A F V T I L S F N A CS D S S A N Q D P N L V A K E S N E V AM K D F G
K T V P V G I E K E D G K F KI S F M V T A Q P Y E I A D S K E N A GY I S M I R Q A V E N E T P V H V F L
K V N T N K I A K V E K A T DD D I R Y FK S V F N K Q E R GE S N K A V S V I PD L A T L N S L F T Q I K N
Q A C G T ST A S S P C I T F R Y P V D G C Y A R AH K MR Q I L L N A GY D C E K Q F V YG N L R A S T G

T C C.V S W V Y H V A IL V S F K N A S G I V E K R I I D P S L F S S G P V T D S A W R A A C T N T S C G S A S V S S Y A N T A G N V Y Y R S P S G S G C S P S P A P S V A S C G F L [0158] L Y D N N Y V N T N C V L N I F S S L S Example 27 Based on the DNA array which carries out the code of the construction N-terminal-region amino acid sequence and C terminal field amino acid sequence of a manifestation plasmid in the Escherichia coli of the production a protein deamidase in the Escherichia coli of the protein deamidase, two sorts of following oligonucleotides were compounded with the DNA synthesis machine (Applied Biosystems), and it considered as the PCR primer.

[0159] array number 7 sense primer: -- 5 -- '-GCGAATTCGCAGTCAGTGTTATTCCTGATC-3' -- array number 8 antisense primer: -- 5' -TAGAATTCTTAAAATCCACAGCTTGCTAC-3' [0160] The PCR reaction was performed under the following conditions using Omnigene Thermal Cycler (Hybaid shrine) by using as mold plasmid p7T-1 which has these primers and a protein deamidase gene.

the <PCR reaction mixture> 10 x PCR reaction buffer solution (Perkin Elmer) 10.0 muldNTP mixed liquor (respectively 2.5 mM and Promega) 8.0 mul20microM Sense primer 2.5 mul20microM antisense primer 2.5 mul distilled water 75.5 T-mul plasmid p71 solution (50microg/(ml)) 1.0 mulTaq DNA polymerase (Perkin Elmer) 0.5 mul [0161]

<PCR reaction condition> stage 1: Denaturation (94 degrees C, 5 minutes) 1 cycle stage 2: Denaturation (94 degrees C, 1 minute) 30 cycles Annealing (55 degrees C, 1 minute)

Expanding (72 degrees C, 1 minute)

Stage 3: Expanding (72 degrees C, 10 minutes) One cycle [0162] From [after carrying out cloning of the DNA fragment of about 0.57 obtained kbs to pCRII (Invitrogen) and checking that a base sequence is right] this plasmid EcoRI processing recovered the DNA fragment of about 0.57 kb. This DNA fragment was inserted in the EcoRI part of expression vector pGEX-1lambdaT (Pharmacia) in Escherichia coli, and the code DNA of the protein deamidase was connected with the C terminal of the code DNA of the glutathione S transferase which pGEX-1lambdaT has in this direction. The manifestation plasmid pN 7-7 in the Escherichia coli of the obtained protein deamidase can make the fusion protein of a glutathione S transferase and the protein deamidase able to discover under a tac promotor's control, and can start the protein deamidase from a fusion protein by Thrombin processing.

[0163] b) The manifestation manifestation plasmid pN 7-7 in the Escherichia coli of the protein deamidase was introduced into Escherichia coli BL21 (Pharmacia), and the transformant was obtained. Moreover, the transformant of Escherichia coli BL21 which has expression vector pGEX-1lambdaT as contrast was also obtained. It cultivated into the cell of the logarithmic growth phase (OD 600= 0.9~1.0) which cultivated and obtained these transformants by 37 degrees C and 200rpm by LB culture medium containing 100microg [/ml] ampicillin on these conditions after adding IPTG of final concentration 0.1 mM for further 4 hours, and the harvest was carried out to it. They are 50 mM Tris-HCl of 1/10 amount of culture medium, and pH8.0/2 mM about a fungus body. It ****(ed) to EDTA and Triton X-100 of final concentration 0.1 mg/ml Egg white lysozyme and 0.1% of final concentration were added, ****** DNA was ****(ed) at 30 degrees C by mild sonication (it is 3 cycles about 10 sec.On and 30 sec.Off) after 15 min neglect, and Cell extract was obtained. To this Cell extract 100microl, Thrombin (1 U/mu l-9 mM sodium phosphate, pH6.5/140 mM NaCl) of 4microl was added, it was left in the room temperature for 16 hours, and the Trombin processing Cell extract was obtained. Moreover, the buffer solution (9 mM sodium phosphate, pH6.5/140 mM NaCl) of 4microl was added, and it considered as contrast of Trombin processing of what performed the same reaction.

[0164] About the obtained sample, the result of having measured protein deamidation enzyme activity is shown in the following tables.

[0165]

[Table 7]

サンプル	形質転換体	Trombin 処理	蛋白質脱アミド活性 (mU/ml)	
			基質: Z-Gln-Gly	基質:カゼイン
1	E. coli BL21/pN7-7	-	30.02	16.10
2	E. coli BL21/pN7-7	+	35.36	19.99
3	E. coli BL21/pGEX-1λT	-	0.00	0.00
4	E. coli BL21/pGEX-1λT	+	0.00	0.00

[0166] Thus, it turns out that the Escherichia coli which has the protein deamidase manifestation plasmid pN 7-7 has discovered protein deamidation activity. The manifestation of protein deamidation activity was not observed by the Escherichia coli which, on the other hand, has expression vector pGEX-1lambdaT considered as contrast. Moreover, they are these samples 12% SDS-polyacrylamide gel electrophoresis was presented and Western analysis using the antibody to the protein deamidase was performed. Consequently, the antibody and the band which reacts were detected in the location of molecular-weight abbreviation 43kDa which has the fusion protein of a glutathione S transferase and the protein deamidase thought in a sample 1, and the band was detected in the sample 2 by the location of molecular-weight abbreviation 20kDa of the protein deamidase other than this band of molecular-weight abbreviation 43kDa. On

the other hand, an antibody and no bands which react were detected in a sample 3 and 4. From these results, it was checked using the protein deamidase gene obtained by this invention that the recombination protein deamidase can be manufactured using Escherichia coli.

[0167] Example 28 Based on the DNA array which carries out the code of the construction N-terminal-region amino acid sequence and C terminal field amino acid sequence of a manifestation cassette in the mold of the manifestation a protein deamidase in the mold of the protein deamidase, two sorts of following oligonucleotides were compounded with the DNA synthesis machine (applied system company), and it considered as the PCR primer.

[0168] array number 9 sense primer: -- 5 -- '-GCGTCGACGCAGTCAGTGTTATTCCTGATC-3' -- array number 10 antisense primer: -- 5' -TAGGATCCTTAAAATCCACAGCTTGCTAC-3' [0169] The PCR reaction was performed like the example 27 by using as mold plasmid p7T-1 which has these primers and a protein deamidase gene. After carrying out cloning of the DNA fragment of about 0.57 obtained kbs to pCRII (Invitrogen) and checking that a base sequence is right, SalI/BamHI processing recovered the DNA fragment of about 0.57 kb from this plasmid. This DNA fragment was inserted in the SalI-BamHI part of manifestation cassette construction vector pY4' (JP,7-123987,A) in mold, and plasmid pD5' was obtained. In this plasmid, it has the DNA array which carries out the code of the fusion protein of the protein deamidase to the monochrome of the Penicilliumcamembertii origin, and diacylglycerol lipase. Furthermore, deletion of the unnecessary base sequence (5'-GTCGAC-3' and this are equivalent to the SalI part introduced in order to connect a protein deamidase gene) which exists in the joint of the protein deamidase was carried out to monochrome and diacylglycerol lipase by site-specific mutation, and the plasmid pD5 was obtained. This plasmid has the DNA array which carries out the code of the fusion protein by which the protein deamidase was connected just behind the processing part (Lys-Arg array, respectively the 3rd from a C terminal, and the 2nd) of the C terminal part of monochrome and diacylglycerol lipase. Moreover, this fusion protein is discovered by control of the promotor of the monochrome of the Penicillium camembertii origin on that upstream and lower stream of a river, and a diacylglycerol lipase gene, and a terminator with mold or yeast. Moreover, it is possible for the discovered fusion protein to be cut by the Lys-Arg part by the protease which a host has, and to start the protein deamidase (JP,7-123987,A).

[0170] b) The plasmid pD5 obtained by the manifestation above a by Penicilluim camembertii of the protein deamidase was introduced into -150 shares of Penicillium camembertii U by co-transformation with the transformation plasmid pH 1 of Penicillium camembertii. The transformant only by pH1 was also obtained as contrast. When the obtained transformant was cultivated and the protein deamidation enzyme activity in a culture filtrate was measured, in the transformant which introduced the plasmid pD5, production of the 10.3 mU/ml (substrate: Z-Gln-Gly) protein deamidase was checked. On the other hand, activity was not detected in the culture filtrate of the transformant only by pH1. In addition, as for the plasmid pH 1 and the transformation method, the detail is indicated by JP,7-123987,A.

[0171] Example 29 It is the plasmid pD5 obtained by manifestation example 28 a in Aspergillus oryzae of the protein deamidase by co-transformation in the transformation plasmid pN3 of Aspergillus oryzae 1.1 shares of Aspergillus oryzae AO (Mol.Gen.Genet., 218, 99-104, 1989) It introduced. The transformant only by pN3 was also obtained as contrast. When the obtained transformant was cultivated and the protein deamidation enzyme activity in a culture filtrate was measured, in the transformant which introduced the plasmid pD5, production of the 4.51 mU/ml (substrate: Z-Gln-Gly) protein deamidase was checked. On the other hand, activity was not detected in the culture filtrate of the transformant only by pN3. In addition, as for the plasmid pN3 and the transformation method, the detail is indicated by JP,7-123987,A. From the result of examples 28 and 29, it was checked using the protein deamidase gene obtained by this invention that the recombination protein deamidase can be manufactured using mold.

[Translation done.]

CLAIMS

[Claim(s)]
[Claim 1] The enzyme which has the operation which carries out deamidation of the amide group in protein.
[Claim 2] The enzyme which has the operation which carries out a direct action to the amide group in protein, and accompanies by them and carries out deamidation of cutting of peptide linkage, and the proteinic bridge formation.
[Claim 3] This enzyme is an enzyme according to claim 1 or 2 characterized by being the microorganism origin.
[Claim 4] The polypeptide which consists of a polypeptide which one piece or two or more amino acid residue have the amino acid sequence by which at least one of deletion, addition, insertion, or the permutations is made, and has the operation to which deamidation of the amide group in protein is carried out in an amino acid sequence given in the array number 6 of an array table.
[Claim 5] The polypeptide which becomes the array number 6 of an array table from the polypeptide which has the amino acid sequence of a publication.
[Claim 6] The nucleotide which carries out the code of the polypeptide which has the activity which carries out deamidation of the amide group in protein.
[Claim 7] The nucleotide which carries out the code of the polypeptide which has the activity which carries out a direct action to the amide group in protein, and accompanies by them and carries out deamidation of cutting of peptide linkage, and the proteinic bridge formation.
[Claim 8] The nucleotide which consists of a nucleotide which carries out the code of the polypeptide which has the activity which consists of a nucleotide chosen from following (a) - (g), and carries out deamidation of the amide group in protein.
(a) In an amino acid sequence given in the array number 6 of a nucleotide and the (b) array table which carries out the code of the polypeptide which has the amino acid sequence of a publication to the array number 6 which is an array table The nucleotide to which one piece or two or more amino acid residue carry out the code of the polypeptide which has the amino acid sequence by which at least one of deletion, addition, insertion, or the permutations is made, (c) In a base sequence given in the array number 5 of a nucleotide and the (d) array table which has the base sequence of a publication for the array number 5 of an array table The nucleotide in which one piece or two or more bases have the base sequence by which at least one of deletion, addition, insertion, or the permutations is made, (e) The gene hybridized under stringent conditions to a nucleotide given in either of above-mentioned (a) - (d), (f) Nucleotide of the nucleotide which has homology in a nucleotide given in either of - (d), and (above-mentioned a) (g) above-mentioned (a) - (f) which degenerates to the nucleotide of any one publication at least.
[Claim 9] The nucleotide which consists of a nucleotide which carries out the code of the polypeptide which has the amino acid sequence of a publication to the array number 6 of an array table.
[Claim 10] The recombination vector characterized by containing the nucleotide of a publication in either of claims 5-9.

[Claim 11] The transformant into which the recombination vector according to claim 10 was made to introduce.
[Claim 12] The manufacturing method of the enzyme which has the operation which carries out deamidation of the amide group in the protein characterized by extracting the enzyme which has the operation which cultivates a transformant according to claim 11, is made to produce the enzyme which has the operation which carries out deamidation of the amide group in protein, and carries out deamidation of the amide group in protein from a culture.
[Claim 13] The recombination polypeptide which cultivates a transformant according to claim 12 and is extracted from this culture and which has the operation which carries out deamidation of the amide group in protein.
[Claim 14] The manufacturing method of the new enzyme characterized by cultivating a microorganism to a nutrition culture medium, making the new enzyme which has the operation which carries out deamidation of the amide group in protein produce, and extracting this enzyme.
[Claim 15] The manufacturing method of the new enzyme which has the operation which carries out deamidation of the amide group in the protein which carries out enzyme production and is characterized by the new thing for which it has the operation which cultivates a microorganism to a nutrition culture medium, carries out a direct action to the amide

group in protein, and accompanies by them and carries out deamidation of cutting of peptide linkage, and the proteinic bridge formation, and for which this enzyme is extracted.
[Claim 16] Claim 14 whose microorganisms are the bacteria classified into SHITOFAGARESU (Cytophagales) or bitter taste chino my SETESU (Actinomycetes), or a manufacturing method according to claim 15.
[Claim 17] Claim 14 whose microorganisms are the bacteria classified into FURABOBAKUTERIACHIE (Flavobacteriaceae), or a manufacturing method according to claim 15.
[Claim 18] Claim 14 as which a microorganism is chosen from a KURISEO bacterium (Chryseobacterium) group, the Flavobacterium (Flavobacteium) group, an en PEDOBAKUTA (Empedobacter) group, the Sphingobacterium (Sphingobacterium) group, an AUREO bacterium (Aureobacterium) group, and a MIROIDESU (Myroides) group, or a manufacturing method according to claim 15.
[Claim 19] The method of embellishing the protein and/or the peptide which are characterized by making the enzyme which has the operation which carries out a direct action to the amide group in protein and a peptide, and accompanies by them and carries out deamidation of cutting of peptide linkage, and the proteinic bridge formation to protein and/or a peptide act.
[Claim 20] The constituent for qualification of the protein and/or the peptide which become considering the enzyme which has the operation which carries out a direct action to the amide group in protein and a peptide, and accompanies by them and carries out deamidation of cutting of peptide linkage, and the proteinic bridge formation as an active principle.
[Claim 21] How to make the enzyme which has the operation which carries out a direct action to the amide group in protein and a peptide, and accompanies by them and carries out deamidation of cutting of peptide linkage, and the proteinic bridge formation act on a vegetable property, an animal protein, and/or a peptide, and to improve the functionality of the protein concerned and/or a peptide.
[Claim 22] How to make the enzyme which has the operation which carries out a direct action to the amide group in protein and a peptide, and accompanies by them and carries out deamidation of cutting of peptide linkage, and the proteinic bridge formation act on the food containing a vegetable property, an animal protein, and/or a peptide, and to improve the functionality of the food concerned.
[Claim 23] How to make the enzyme which has the operation which carries out a direct action to the amide group in protein and a peptide, and accompanies by them and carries out deamidation of cutting of peptide linkage, and the proteinic bridge formation act on the rough raw material containing a vegetable property, an animal protein, and/or a peptide, and to improve the extraction efficiency of the protein from the rough raw material concerned, and/or a peptide.
[Claim 24] How to control the reaction of transglutaminase by the enzyme which has the operation which carries out a direct action to the amide group in protein and a peptide, and accompanies by them and carries out deamidation of cutting of peptide linkage, and the proteinic bridge formation.

[Translation done.]

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] It is drawing showing the culture progress by the lactose culture medium of KURISEO bacterium GUREUMU (Chryseobacterium gleum) JCM2410.
[Description of Notations]
** pH fluctuation is shown, x shows 660nm absorbance (turbidity) fluctuation of culture medium, fluctuation of protease activity when ** makes casein a substrate is shown, fluctuation of protein deamidation enzyme activity when - makes Z-Gln-Gly a substrate is shown, and a black trigonum shows fluctuation of the protein deamidation enzyme activity when making casein into a substrate.
[Drawing 2] It is drawing showing the optimal pH of the protein deamidase obtained from KURISEO bacterium GUREUMU (Chryseobacterium gleum) JCM2410.
[Drawing 3] It is drawing showing the optimum temperature of the protein deamidase obtained from KURISEO bacterium GUREUMU (Chryseobacterium gleum) JCM2410.
[Drawing 4] It is drawing showing the pH stability of the protein deamidase obtained from KURISEO bacterium GUREUMU (Chryseobacterium gleum) JCM2410.
[Drawing 5] It is drawing showing the temperature stability of the protein deamidase obtained from KURISEO bacterium GUREUMU (Chryseobacterium gleum) JCM2410.
[Drawing 6] It is drawing showing the culture progress by the lactose culture medium of KURISEO bacterium indolo GENESU (Chryseobacterium indologenes) IFO14944.
[Description of Notations]
** pH fluctuation is shown, x shows 660nm absorbance (turbidity) fluctuation of culture medium, fluctuation of protease activity when ** makes casein a substrate is shown, fluctuation of protein deamidation enzyme activity when - makes Z-Gln-Gly a substrate is shown, and a black trigonum shows fluctuation of the protein deamidation enzyme activity when making casein into a substrate.
[Drawing 7] It is drawing showing the culture progress by the lactose culture medium of the KURISEO bacterium MENINGOSE petit cam (Chryseobacterium meningosepticum) IFO12535.
[Description of Notations]
** pH fluctuation is shown, x shows 660nm absorbance (turbidity) fluctuation of culture medium, fluctuation of protease activity when ** makes casein a substrate is shown, fluctuation of protein deamidation enzyme activity when - makes Z-Gln-Gly a substrate is shown, and a black trigonum shows fluctuation of the protein deamidation enzyme activity when making casein into a substrate.
[Drawing 8] It is drawing showing the culture progress by the lactose culture medium of KURISEO bacterium ballast CHINAMU (Chryseobacterium balustinum) IFO15053.
[Description of Notations]
** pH fluctuation is shown, x shows 660nm absorbance (turbidity) fluctuation of culture medium, fluctuation of protease activity when ** makes casein a substrate is shown, fluctuation of protein deamidation enzyme activity when - makes Z-Gln-Gly a substrate is shown, and a black trigonum shows fluctuation of the protein deamidation enzyme activity when making casein into a substrate.
[Drawing 9] It is drawing showing the result of the SDS-polyacrylamide gel electrophoresis of the purification protein deamidase of an example 9, and the sample of each step of purification.
[Drawing 10] It is drawing showing the ammonia of an example 11, or the isolation pattern of ammonium.
[Description of Notations]
** shows the result at the time of adding the protein deamidase, and a black trigonum shows the result of a blank.
[Drawing 11] It is drawing showing the dispersibility of the deamidation gluten of an example 12.
[Description of Notations]
A black rectangular head shows the result of the gluten which carried out protein deamidase processing, and - shows an

unsettled result.
[Drawing 12] It is drawing showing the solubility of the deamidation gluten of an example 12.
[Description of Notations]
A black rectangular head shows the result of the gluten which carried out protein deamidase processing, and - shows an unsettled result.
[Drawing 13] It is drawing showing the ammonia of an example 18, or the isolation pattern of ammonium.
[Description of Notations]
O The result at the time of adding the protein deamidase is shown, and - shows the result of a blank.
[Drawing 14] It is drawing showing the result of the SDS-polyacrylamide gel electrophoresis of the deamidation casein of an example 18.
[Drawing 15] It is drawing showing the calcium solubility of the deamidation casein of an example 19.
[Description of Notations]
A black rectangular head shows the result of the casein which carried out protein deamidase processing, and - shows an unsettled result.
[Drawing 16] It is drawing showing the result of the SDS-polyacrylamide gel electrophoresis at the time of adding the protein deamidase during the transglutaminase reaction of an example 23.
[Drawing 17] It is drawing showing the result of the SDS-polyacrylamide gel electrophoresis at the time of processing the deamidation casein of an example 23 by transglutaminase.
[Drawing 18] It is drawing showing the part acidity and the solubility of deamidation casein of an example 25.
[Description of Notations]
A black rectangular head shows the result of the casein which carried out protein deamidase processing, and - shows an unsettled result.

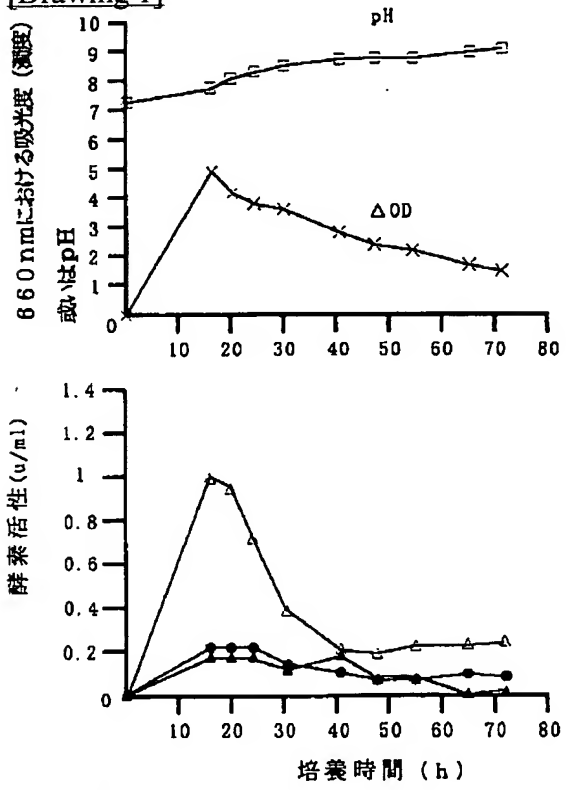
[Translation done.]

* NOTICES *

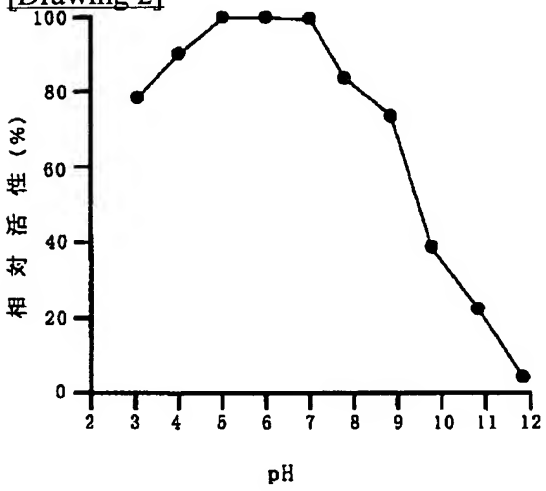
1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DRAWINGS

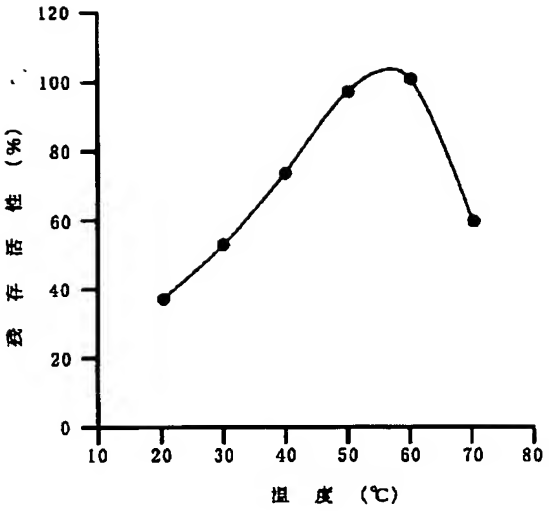
[Drawing 1]



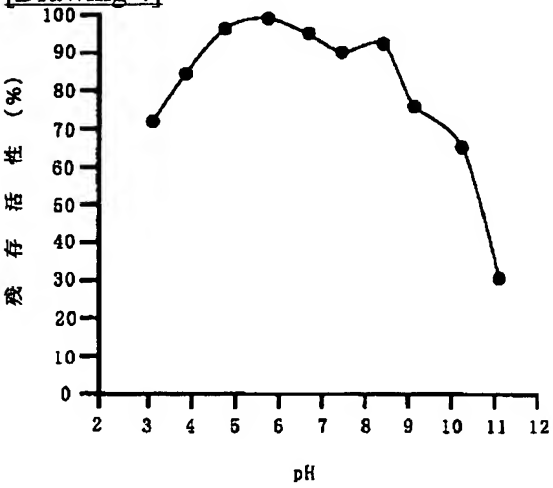
[Drawing 2]



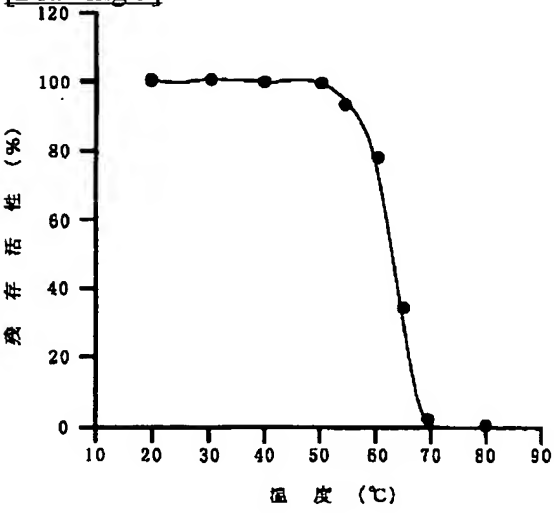
[Drawing 3]



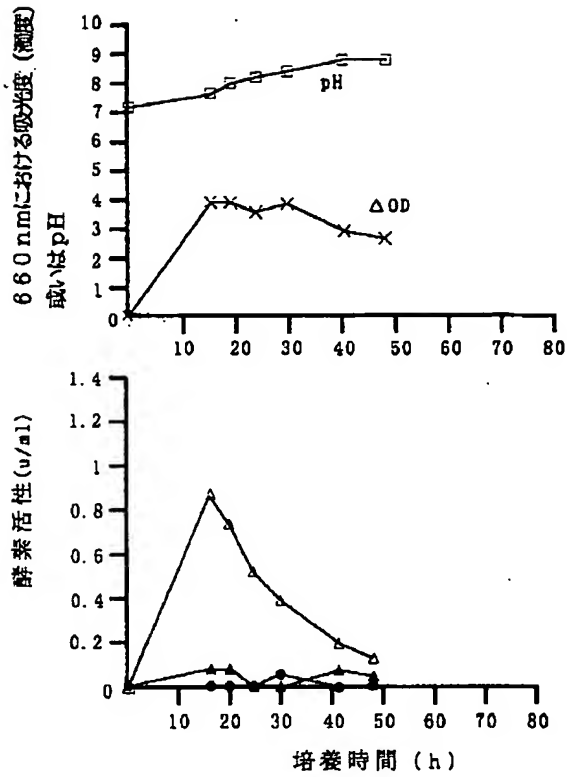
[Drawing 4]



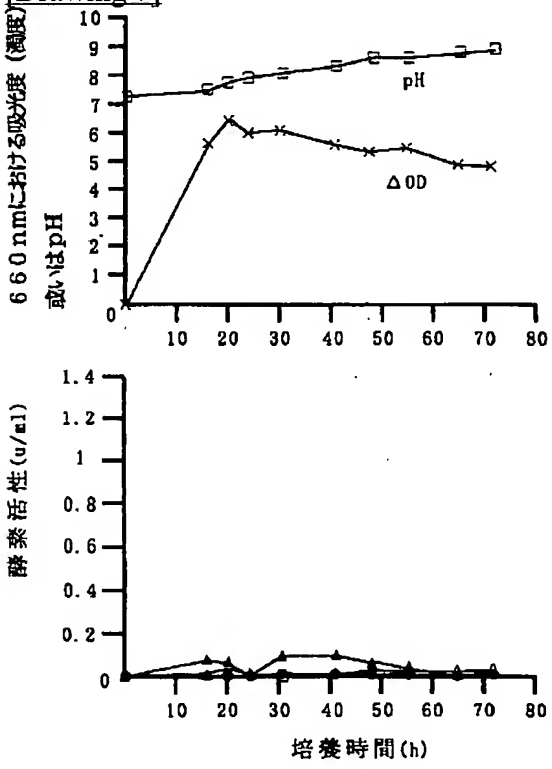
[Drawing 5]



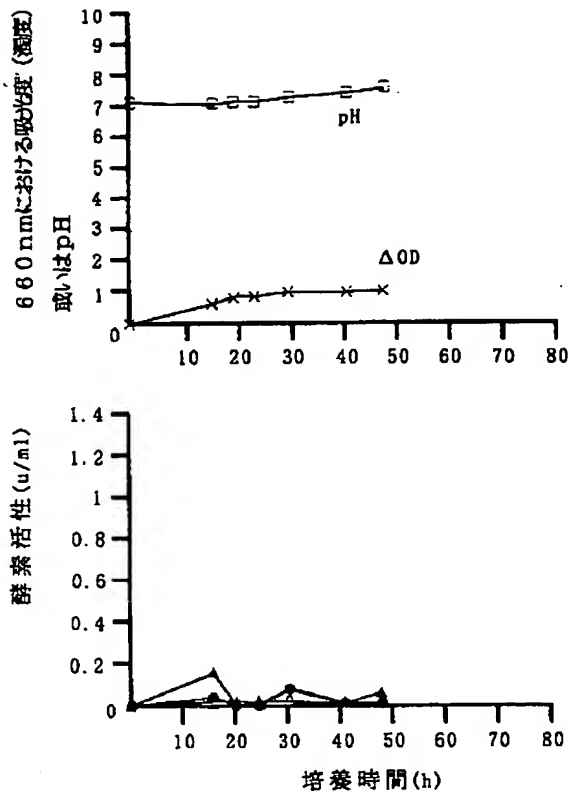
[Drawing 6]



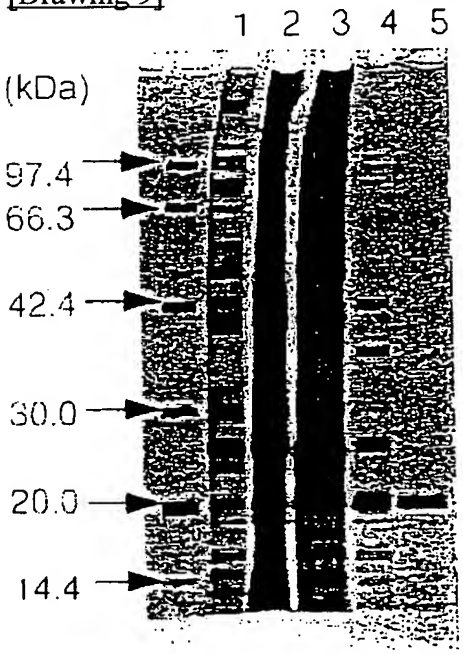
[Drawing 7]



[Drawing 8]



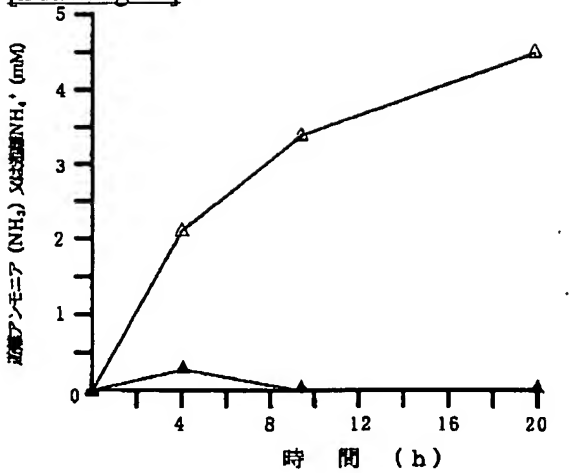
[Drawing 9]



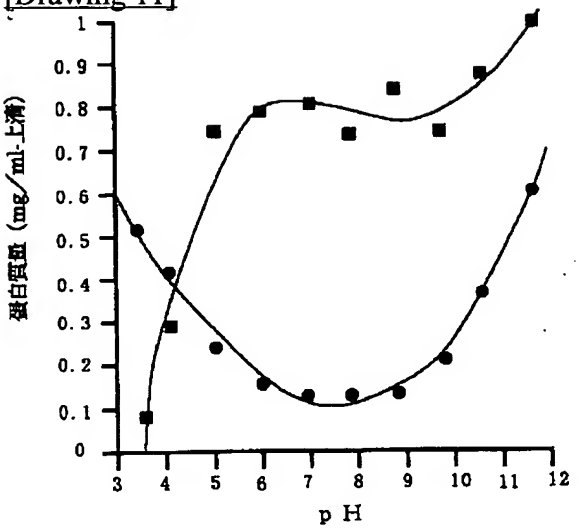
レーン

1. 培養液上清
2. UF濃縮液
3. 2.0M NaCl
4. フェニルセファロース
5. セファクリルS-100

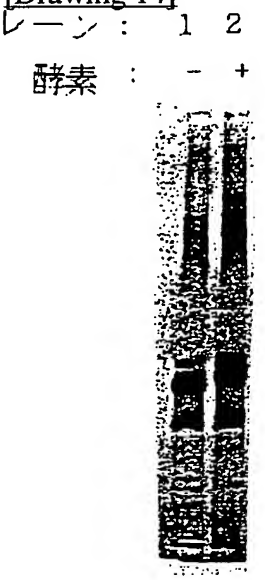
[Drawing 10]



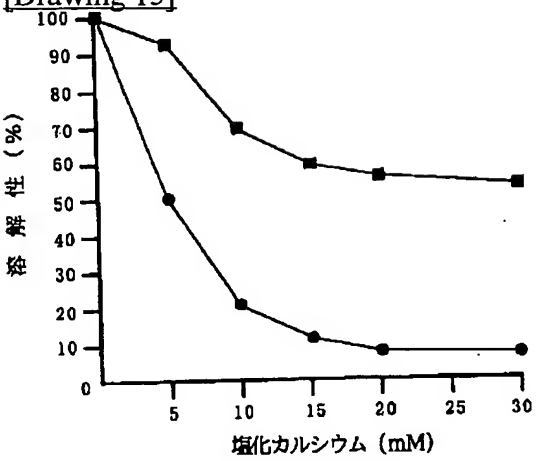
[Drawing 11]



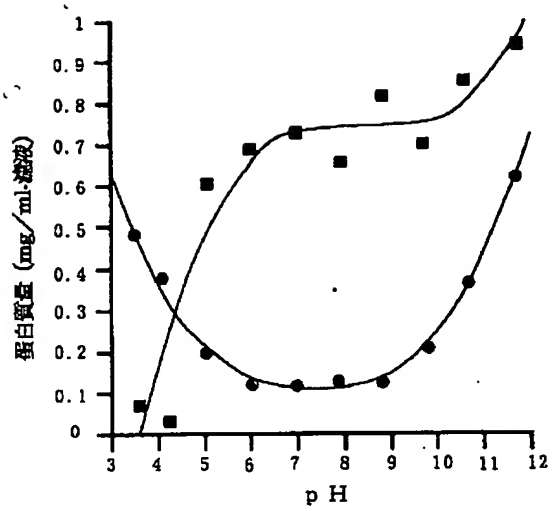
[Drawing 14]



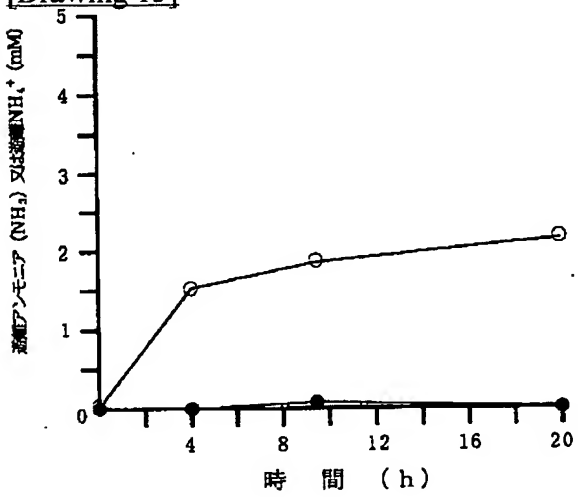
[Drawing 15]



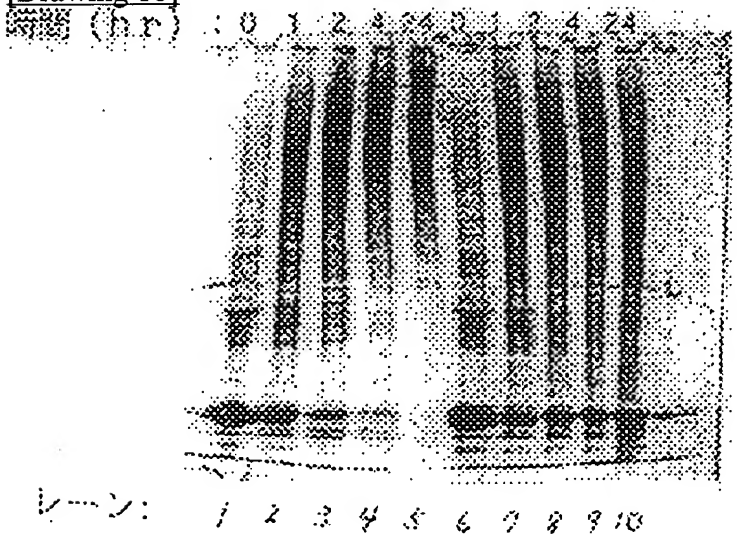
[Drawing 12]



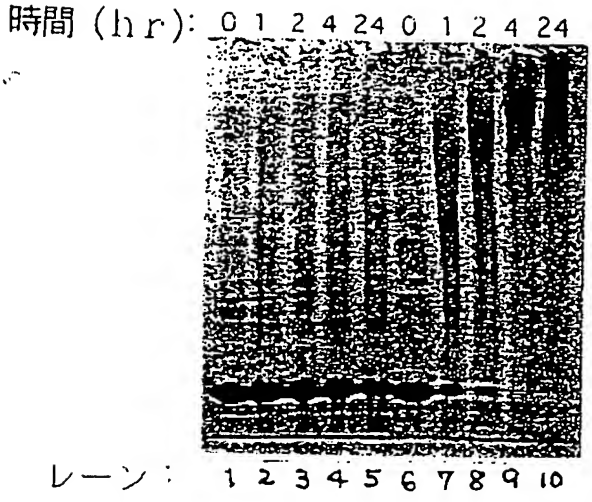
[Drawing 13]



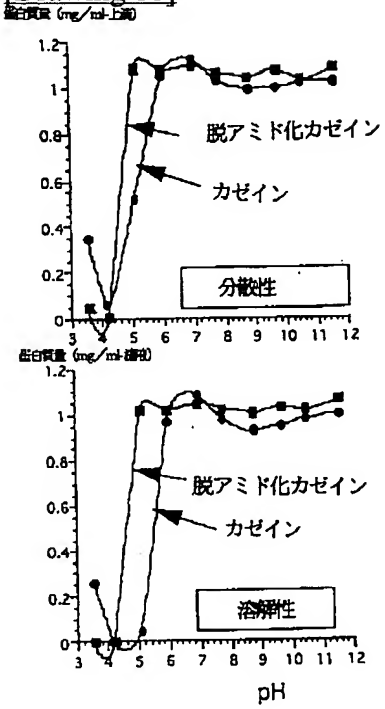
[Drawing 16]



[Drawing 17]



[Drawing 18]



[Translation done.]